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الجامعة الأردنيـــة
كلية الدراسات العليا

التاريـــخ: ٢٨/١٢/٢٠١١

نموذج رقم (١٦)
اقرار والتزام بالمعايير الأخلاقية والأمانة العلمية وقوانين الجامعة الأردنية وأنظمتها وتعليماتها لطلبة الدكتوراه

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عنوان الاطروحة: Assessment of Antimicrobial and Antioxidant Activity of Essential Oils Extracted from some selected Aromatic Jordanian medicinal plants

اعلن بأنني قد التزمت بقوانين الجامعة الأردنية وأنظمتها وتعليماتها وقراراتها السارية المفعول المتعلقة باعداد اطروحات الدكتوراه عندما قمت شخصيا" باعداد اطروحتي وذلك بما ينسجم مع الأمانة العلمية وكافة المعايير الأخلاقية المتعارف عليها في كتابة الأطروحات العلمية. كما أنني أعلن بأن اطروحتي هذه غير منقولة أو مستلة من أطاريح أو كتب أو أبحاث أو أي منشورات علمية تم نشرها أو تخزينها في أي وسيلة اعلامية، وتأسيسا" على ما تقدم فانني أتحمل المسؤولية بأنواعها كافة فيما لو تبين غير ذلك بما فيه حق مجلس العمداء في الجامعة الأردنية بالغاء قرار منحي الدرجة العلمية التي حصلت عليها وسحب شهادة التخرج مني بعد صدورها دون أن يكون لي أي حق في التظلم أو الاعتراض أو الطعن بأي صورة كانت في القرار الصادر عن مجلس العمداء بهذا الصدد.

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ASSESSMENT OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OILS EXTRACTED FROM SOME SELECTED AROMATIC JORDANIAN MEDICINAL PLANTS

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This Dissertation was Submitted in Partial Fulfillment of the Requirements for the Doctor of Philosophy Degree in Biology

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II

COMMITTEE DECISION

This Dissertation (Assessment of Antimicrobial and Antioxidant activity of Essential Oils Extracted from Some Selected Aromatic Jordanian Medicinal Plants) was Successfully Defended and Approved on 9/8/2011

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DEDICATION

TO MY BELOVED PARENTS

MY WIFE & SONS

MY SISTER & BROTHERS

TO MY FRIENDS & COLLEAGUES

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BHA	Butylhydroxyanisole
BHT	Butylhydroxytoluene
DMSO	Dimethyl sulfoxide
DPPH	Diphenylpicrylhydrazyl
HAT	Hydrogen Atom Transfer
MIC	Minimum Inhibition Concentrations
NO	Nitric Oxide
OD	Optical density
ORAC	Oxygen radical absorbance capacity
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SET	Single Electron Transfer
SFE	Supercritical fluid extraction
SOD	Superoxidedismutase

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ABSTRACT

This study aimed to screen the antimicrobial, antioxidant and chemical composition of 10 aromatic Jordanian plants essential oils. The essential oil was extracted from plant that collected from different region in Jordan using steam distillation method.

In order to study the antimicrobial activity of plant essential oils the well diffusion method and determination of MIC were performed. All the essential oils possessed antimicrobial activity against all tested microorganisms. It was detected that most plant essential oils possessed higher activity against microorganisms coparing to standard antibiotic and antifungal. *T. capitatus* showed the lowest MIC against bacteria (2.5 - 5µg/ml) and fungi (10-20µg/ml) followed by *M syriaca*.

The effect of the essential oils on fungal spore germination was studied all essential oils inhibit the spore germination at concentration dependent manner. There was more than 90% inhibition of spore germination at the MIC of each essential oils. No spore germination was observed at concentration higher than MIC.

The antioxidant activities of the essential oils were performed using the following methods: DPPH scavenging, NO scavenging, superoxide scavenging, lipid peroxidation, hydroxyl radical scavenging and reducing power. All the essential oils possess antioxidant activity on concentration dependent manner.

The chemical composition of the essential oils was determined using GC-MS analysis method. Most the constituent of the essential oils were identified and the ratio of each compound was recorded. The result showed that there was direct relationship between the chemical composition of the oil and the antioxidant and antimicrobial activity. For example, *T. capitatus* and *M. syriaca* showed the lowest MIC and they contain high amount of thymol and carvacrol compound that have antimicrobial activity.

INTRODUCTION

Introduction

The use of plants in the treatment of many diseases is an ancient practice. People of all continents have long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants, these plants are still widely used in ethnomedicine around the world (Wang *et al*., 2010). Historically, therapeutic results have been mixed; quite often cures or symptom relief resulted and poisonings occurred at a high rate also. Currently, of the one-quarter to one-half of all pharmaceuticals having higher-plant origins, very few are intended for use as antimicrobials, since we have relied on bacterial and fungal sources for these activities. Since the advent of antibiotics in the 1950s, the use of plant derivatives as antimicrobials has been virtually nonexistent (Cowan, 1999).

In recent years there is a much focus on replacing synthetic food additives which might have adverse effects with those of plant-based natural ones (Paradiso *et al*., 2008). The increasing uses of herbal products demand extra attention with particular focus on their safety, effectiveness and drug interactions. Over the last few decades, a substantial body of scientific evidence is available demonstrating wide range of pharmacological and nutraceutical activities of medicinal herbs (Celiktas *et al*., 2007). These include antioxidant, anticancer, anti-inflammatory activities.

The infectious diseases mainly caused due to microbial contamination of foods are becoming a major problem in the world, particularly in the developing societies (Burt, 2004; Hussain *et al*., 2008). The consumption of microbes-infected foods is a serious challenge and threat for the health of the consumers (Hussain *et el.,* 2008).The microbial growth in foods not only leads to decrease the nutritive and organoleptic value of food commodities, nevertheless it generates several toxins that are harmful for the health of humans (Celiktas *et al*., 2007). Recently, the essential oils and herbs-

derived extracts are gaining much recognition as a potential source of natural and safer antioxidants and bioactives (Burt, 2004; Celiktas *et al.*, 2007;; Hussain *et al.*, 2008).

Due to the bactericidal and fungicidal properties of essential oils, their pharmaceutical and food uses are becoming increasing as alternatives to synthetic chemical products to protect the ecological equilibrium (Burt, 2004). On the other hand, for perfume industry the oils produced by extraction with lipophilic solvents and sometimes with supercritical carbon dioxide are more appealing (Donelian *et al.*, 2009).

Arabs were the first who developed the techniques for obtaining essential oil from the naturally occurring organic materials (Saeed, 1989). Arab physician, Avicenna, designed the protocol to extract the essential oil from the flowers by distillation in the tenth century. He isolated the perfume in the form of oil or attar from the rose flowers and produced rose water. Therefore, the first description of rose water had been reported by an Arab historian, Ibn-e-Khulduae.

Researchers are interested in the biological activity, especially antimicrobial and antioxidant activity of plant extracts and plant products for many reasons: First it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians; severals are already being tested in humans (Cowan, 1999). Second the presence of antibiotic resistant microorganisms that may resist several types of antibiotic. Third the effective life span of many antibiotics is limited. Finally the public is becoming increasingly aware of problems with the over prescription and misuse of conventional antibiotics.

Finding and identifying of naturally originated antioxidant compounds have become important issue knowing that free radicals, e.g., superoxide ions ($O_2^{\cdot}$), hydroxyl radicals ($OH^{\cdot}$) and non-free radical compounds, are responsible for lipid peroxidation (deterioration) in foods (Robinson, *et al*., 1997) and for various diseases such as malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes and cancer (Alho and Leinonen, 1999). It has been reported that some synthetic antioxidant compounds such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), commonly used in processed foods, have side effects due to their possible activity as promoters of carcinogenesis (Rice-Evans, *et al*., 1997).

Very few investigations were made on the antimicrobial and antioxidant activities of Jordanian medicinal plants essential oils and there biologically active constituents. This study aims to verify the chemical composition, antioxidant and antimicrobial activity of some selected aromatic medicinal plants essential oils.

CHAPTER ONE

LITERATURE REVIEW

Role of medicinal plants in medicine

The old saying "Eat an apple a day leave the doctor a way" describes the importance of using plant in medicine. In addition, medicinal plants were used in a wide range for treating disease from ancient times until know, due to believe of their safety (Dhawan 2003). In the last few decades many pharmacological investigations were carried out to identify new drugs or to find new lead structures for the development of novel therapeutic agents for the treatment of human diseases such as cancer and infectious diseases (Newman *et al.* 2003).

The clinical, pharmaceutical, and economical values of medicinal plants are still growing. Based on current research, medicinal plants will continue to play an important role as a health aid .The use of herbal medicines in Asia represents a long history with several applications against various diseases (Draipandiyan, *et al*. 2006). Although there is a large number of estimated plant species, the biological activity of only 6 % has been studied and about 15% have been phytochemically investigated (Goyal, *et al*. 2007).

Until the nineteenth century medicinal plants were the main source of products used to maintain well being, when the German chemist Friedrich Wöhler in 1828, attempting to prepare ammonium cyanate from silver cyanide and ammonium chloride, accidentally synthesized urea. This was the first organic synthesis in history and revealed a new area of the synthetic compounds (Mendonça 2006). Nowadays, medicinal plants are back into use due to the ineffectiveness of conventional medicines such as antibiotics (Husain, *et al*. 2007).

Recently, the use of plants as medicines has involved the isolation of active compounds, beginning with the isolation of morphine from opium in the 19th century. Drug discovery from medicinal plants led to isolation of early drugs such as cocaine, codeine, digitoxin, and quinine, in addition to morphine, of which some are still in use (Balunas and Kinghorn 2005). Furthermore, some medicines have been derived from medicinal plants like aspirin, ephedrine, reserpine, artemisinin, taxol, hypericin and silymarin (Singh 2006, butler 2004).

Jordanian Medicinal Plants

Due to geographical and climatic circumstances there is a great variation in wild plant of Jordan. More than 2000 plant species belonging to about 700 genera were identified. Among these plants, there are more than 485 medicinal species from approximately 99 plant families (Oran and Al-Eisawi, 1998; Afifi and Abu-Irmaileh, 2000). As in most countries medicinal plants are used widely in Jordan and have become an element of indigenous systems in Jordan. These resources are usually regarded as part of a culture's traditional knowledge (Hudaib *et al*., 2008).

Few check lists were published concerning the Jordanian medicinal plants. A checklist prepared by Oran and Al- Esawi (1998) included a number of medicinal plant species that have many ethnomedical uses. A number of 363 medicinal plant species belongs to 263 genera and 86 famililes was recorded. Another study by Al-Khalil (1995) reported 118 medicinal plants species belonging to 49 families. Hudaib *et al*. (2008) identified Fifty-eight plants that are still being used in traditional practice in Mujib.

A survey of traditional drugs consumed in Jordan indicated that there are 236 traditional drugs used for the treatment of different diseases originated from plants (Lev and Amar, 2002). Many studies investigated the ant oxidant (Al- Mustafa and Al – Thunibat, 2008), antidiabetic (Hamdan and Afifi, 2004), anticancer (Abuharfeil *et al.*, 2000) and antimicrobial activities of different extracts from these plants (Mahasneh and El-Oqlah, 1999).

Processing of Medicinal Plants

Due to the increased use of medicinal plants as an important resource for production of medications and food, there is a large demand for production and processing of plant products as crude materials for several industries. Processing of medicinal plant products need to follow a standardized quality arrangement. The processing of medicinal plant is an important step for good quality of plant products. Monitoring should be done at steps in production process (Groot and Roest 2006).

Essential oils

Essential oils are defined as the volatile oils obtained usually by the steam distillation of plants. From the view point of practical applications, these materials may be defined as odoriferous bodies of an oily nature, obtained almost exclusively from vegetative organs: flowers, leaves, barks, woods, roots, rhizomes, fruits, and seeds (Celiktas *et al.*, 2006). They composed of a mixtures of fragrant substances or mixtures of fragrant and odorless substances. A fragrant substance is a chemically pure compound, which is volatile under normal conditions and which owing to its odor can be used in medication (Gunther, 1952).

Essential oils are generally liquids composed of complex and highly variable mixture of constituents that belong to two chemical groups: terpenoids and phenolic compounds (Guenther, 1952). Their composition can be expressed by the general formula $(C_5H_8)_n$ for monoterpene, $(C_{10}H_{16})_n$ n=2 for diterpene and $(C_{20}H_{32})$ and $(C_{15}H_{24})_n$ for sesquiterpenes; n is greater than 2. Although essential oils are comprised of many types of compounds, the major ones are monoterpenes (Seigler 1998).

Sources of essential oils

Different aromatic plants generally produce essential oils in almost all of their organs such as flowers, buds, stems, leaves, fruits, seeds and roots; however, flowers fruit and leaves are the richest. These essential oils accumulate in secretary cells, cavities, channels, and epidermic cells (Anwar *et al.,* 2009). The plant raw material from which essential oils are manufactured may be fresh, partially dehydrated or dried (Hussain *et al*., 2008).

The most important methods that are still used for extraction of essential oils are steam distillation and hydrodistillation (Sokovic and Van Griensven, 2006). Essential oils can be extracted using other methods such as superartificial fluid (SFE), using liquid carbon dioxide, microwave extraction, and low or high pressure distillation employing boiling water or hot steam (Bousbia, *et al*., 2009).

Due to the increase in using of essential oils in various sectors including pharmacy, perfumery, cosmetics, aromatherapy, and food and beverages industry there is an extensive need of reliable methods for analyses of essential oils. These requirements have been satisfactorily fulfilled by Gas chromatography (GC) and The combination of gas chromatography and mass spectrometry (GC-MS) techniques

(Burt, 2004). GC-MS allows rapid and reliable identification of essential oils components (Yadegarinia *et al*., 2006).

Factors Affecting Essential Oil Accumulation

Factors that determine the composition and yield of the essential oil obtained are numerous. In some instances it is difficult to segregate these factors from each other, since many are interdependent and influence one another (Terblanche, 2000). These variables may include seasonal and maturity variation, geographical origin, genetic variation, growth stages, part of plant utilized and postharvest drying and storage (Anwar *et al.,* 2009).

Seasonal and maturity variations

These two factors are interlinked with each other, because the specific ontogenic growth stage will differ as the season progresses. There are many reports in literature regarding the variation in the chemical profile of essential oils from various plants collected during different seasons (Celiktas *et al*., 2007; Hussain *et al*., 2008). The essential oils yields varied considerably from month-to-month and was also influenced by the micro-environment (sun or shade) in which the plant was growing (Juliani *et al.,* 2002). Pala-Paul *et al*. (2001) reported month-to-month variations in the essential oil composition and yield of *Santolina rosmarinifolia,* which could be attributed to precipitation and temperature. Results obtained by Badi *et al.*, (2004) for *Thymus vulgaris* (thyme), also indicated that timing of harvest is critical to both yield and oil composition.

McGimpsey *et al*. (2006) reported seasonal variation in the essential oil composition and yield from naturalized *Thymus vulgaris* collected from Newzeland. The higher essential oil yield from *Thymus vulgaris* was reported in spring and over the nine month harvesting period, thymol was found to vary from 31.5-52.4% (Atti-

Santos *et al*., 2004). With respect to the harvesting time, the thyme essential oils were richer in oxygenated compounds in the spring, followed by summer, autumn and winter (Atti- Santos *et al*., 2004). The maximum yields and phenol content of the oil from naturalized thyme were exhibited from crop harvested after flowering in December (McGimpsey *et al.*, 2006). Guedes *et al*. (2004) determined the amount and composition of the essential oil from leaves of *Hypericum androsaemum* L. cultivated in Arouca (Portugal) harvested during one year at intervals of two months and reported considerable variation in the yield and chemical composition in the essential oils.

Many reports are available in the literatures regarding the variation in the yield and quality of essential oils with respect to maturity stages (Anwar *et al*., 2009). Chalchat *et al*. (1995) reported that the oil yield of young *Tagetes minuta* plants, before flowering, was high, compared to that of plants in early or full flowering stages. However, Graven *et al.* (1990) pointed out that higher *Artemisia afra* oil yields were obtained during anthesis and early seed onset, as opposed to harvesting earlier or later in the reproductive period. In contrast, Marotti *et al*. (1994) found that higher fennel oil yields (*Foeniculum vulgare*) were obtained at ripe seed stage and high limonene contents were a feature of oils obtained during earlier ontogenic stages, whilst *(E)*-anethole was more prominent in the late seed stage. *Origanum vulgare* ssp. *hirtum* has been known to produce less essential oil during the cool and wet vegetative period, and more oil during the warmer and drier flowering stage (Skoula and Harborne, 2002). After flowering, the oil yield decreased, as the leaves aged and became drier. In addition, thymol and/or carvacrol contents were found to decrease during autumn.

Frizzo *et al*. (2004) studied the variation in the chemical composition of the main volatile constituents of hydrodistilled and coldpressing peel oils of two Brazilian mandarin cultivars, with respect to seasons. These results indicate that essential oil of different qualities can be obtained according to the harvest time of the plant (Asghari *et al*., 2002). The chemical composition of *Teucrium orientale* L. var. *orientale* essential oils with respect to harvesting stages is also studied previously by Yildirim *et al*. (2004). Mohammadreza (2008) reported the variation in the quality and quantity of the essential oil obtained from *Artemisia annua* at different developmental growth stages including pre-flowering, flowering and post-flowering.

Goodner *et al.* (2006) investigated the chemical intraspecific variability among plants of *Thymus hyemalis* L. and Spanish *T. vulgaris* subsp. *vulgaris* shrubs.

Geographical variation

There are many reports in the literature showing the variation in the yield and chemical composition of the essential oil with respect to geographical regions (Celiktas *et al.,* 2007). Chalchat *et al*. (1995) reported variations in the yield and chemical profile of essential oils from *Mentha longifolia* (L.) and *Tagetes minuta* populations, collected from different geographical locations, respectively. Such differences could be linked to the varied soil textures and possible adaption response of different populations, resulting in different chemical products being formed, without morphological differences being observed in the plants (Hussain *et al*., 2008).

Altitude seems to be another important environmental factor influencing the essential oil content and chemical composition (Vokou *et al*., 1993). The yield and chemical composition of essential oils from *Origanum vulgare* ssp. *hirtum* essential oils from twenty three localities, scattered all over Greece were varied significantly (Vokou *et al*., 1993).

Climatic factors such as heat and drought were also related to the essential oil profiles obtained (Milos *et al*., 2001). Moreover, the preference of the plant for these conditions suggest that genetic make-up of the plant, rather than the soil-type in which it is growing, should have a greater influence on the chemical profile of the oil produced (Graven *et al*., 1990; Milos *et al*., 2001).

Genetic variation

Genotype is typically defined as “the genetic make-up of an organism, as characterized by its physical appearance or phenotype”, while chemotype is generally defined as “a group of organisms that produce the same chemical profile for a particular class of secondary metabolites”. Variations in chemical profiles were observed from oils produced from specimens from the same population and location, demonstrating the presence of different chemotypes within this species (Ahmad *et al.*, 2006). Genetic makeup of the plant is one of the most important contributors to their essential oil composition (Graven *et al.*, 1990). Ahmad *et al*. (2006) reported that the chemical composition of essential oils of Malta (*Citrus sinensis*), Mousami (*C. sinensis*), Grapefruit (*C. paradisi*) and Eureka lemon (*C. limon*) varied significantly, which may be due to the difference in their genetic makeup.

Other factors affecting yield and composition of essential oil

Other factors which affect the growing plants thus leading to variations in oil yield and composition, include part of plant used (Chalchat *et al*., 1995); post harvest drying; length of exposure to sunlight, availability of water, height above sea level, plant density, time of sowing and the presence of fungal diseases and insects (Graven *et al.*, 1990). The oil composition and yield may also change as a result of the harvesting methods used, the isolation techniques employed, the moisture content of the plants at the time of harvest and the prevailing steam distillation conditions.

Bonnardeaux (1992) investigated the oil composition and yield of different plant parts of basil (*Ocimum basilicum*) and found that lower oil yields were obtained from the distillation of basil with shorter flower spikes than from plants with long flower spikes. Santos-Gomes and Fernandes-Ferreira (2001) reported variation in the chemical composition of the essential oils from stems, leaves and flowers of *Salvia officinalis* L. plants harvested in northern Portugal. Significant variations in the main components of the essential oils from shoots sampled over the year were recorded at two different sites in Northern Portugal.

Postharvest drying of material is an accepted practice in the production of essential oils (Whish and Williams, 1996). Drying methods include exposure to natural air in the shade, sun-drying, as well as drying by blowing warm air over the material (Soysal and Oztekin, 2001). Postharvest drying is thought to improve oil yield and accelerate distillation, by improving heat transfer, in addition to providing increased loading capacity, due to loss of plant moisture. Further advantages include the reduction of microbial growth and the inhibition of some biochemical reactions in dried material (Soysal and Oztekin, 2001). However, some amount of the oil may be lost during such post harvest treatment due to volatilization and mechanical damage to oil glands.

Essential oil components (including terpenoids) are usually present in the free form, but may also be bound to sugar moieties, usually mono- or disaccharides (Skoula and Harborne, 2002). Investigations into the essential oil composition often entail air-drying of the leaves prior to distillation, although no specific reasons were given for this course of action. In some plants, such as *Eucalyptus* species and tea tree (*Melaleuca alternifolia*), postharvest drying, with leaves kept intact on the branches, resulted in an increased oil yield (Whish and Williams, 1996). This has been shown

to be a result of continued postharvest metabolic activity within the plant, perhaps during which oil is moved from the stem to the leaves (Whish and Williams, 1996). Peppermint *(Mentha piperita*) is routinely cut and allowed to partially dry (35% moisture) in the field, during the production of peppermint oil. This practice is known to affect the chemical composition of the oil. The essential oil exhibited considerable chemical transformation in its monoterpenoids as leaves were dried by three different methods (Asekun *et al*., 2007). In contrast to above reports, Demir *et al.* (2004) found no significant differences in the oil yield of fresh bay leaves (*Laurus nobilis*) compared to that of leaves dried at 40, 50 and 60 °C, as well as in sun and shade conditions. However, the oil composition of the dried material differed significantly from that of the fresh material. Baritaux *et al.* (1992) suggested that essential oil losses from dried and stored basil (*Ocimum basilicum*) were mainly due to evaporation, whilst hydrolysis of glycosides during distillation was responsible for an increase in oxygenated monoterpenes.

It was reported that the day light directly influenced the amount of specific chemical components in the essential oils. During a small photoperiod, menthofuran was noted to be the major monoterpene in all of the leaves, while menthol and menthone were present in low concentrations. The conversion of menthone is accelerated at lower (cold) night. In contrast, the maturity of herbage was accelerated at cold nights and it was understood that the less mature, upper parts of plants contained more menthone than the matured parts of (lower half) the plant (Simon *et al.,* 1990). The essential oils yield can be enhanced by adjusting elements of growing system, for instance, plant density, planting date, fertilizer application, harvest date, water supply, and crop protect ion. For some species, for example, peppermint, a part of the oil yield has to be sacrificed to guarantee the required oil quality (Clark and

Menary, 1979). Moreover, monoterpene accumulation in peppermint is determined by the rate of its biosynthesis. Hence, efforts to enhance production of this species should focus on the enzymes, genes, and cell differentiation processes which are responsible for regulation of monoterpene biosynthesis (Gershenzon *et al.*, 2000).

The oil yield and oil quality of three different mint species were compared under different Hungarian and Finnish growing conditions (temperature and light), the oil yield were higher in Hungarian than those of Finnish, however, the menthol percentage of volatile oil was found to be higher in Finnish than that of Hungarian (Aflatuni *et al.,* 1999). Typical peppermint oil has about 50% of menthol, 20% of menthone and 8% of ester (Murray *et al.,* 1988). Flower oil contains much higher menthofuran and pulegone than leaves oil and more than 50% of the flower oil may be composed of menthofuran and pulegone, with less than half of the oil consists of menthol and menthone (Murray *et al.,* 1988). During a long photoperiodic treatment or in nature, the oil with high menthyl acetate was formed in the oldest leaves only, after the accumulation of menthol (Voirin *et al.* 1990).

Antioxidant activity of essential oils

It has long been recognized that naturally occurring substances in higher plants have antioxidant activity. Recently, there is a growing interest in oxygen-containing free radicals in biological systems and their implied roles as causative agents in the etiology of a variety of chronic disorders. Accordingly, attention is focused on the protective biochemical functions of plant originated antioxidants (Halliwell, 1997). The plant essential oils and their constituent were widely screened for their antioxidant activity.

Kivrak *et al*. (2009) analyzed the essential oil of *Salvia potentillifolia* by GC and GC–MS. Totally, 123 components were detected in both hydrodistilled and steam-distilled oils, α and β-pinenes being the major compounds. The antioxidant activities were determined by using complementary tests, namely, 1, 1-diphenyl, 2-picryl hydrazyl (DPPH) radical-scavenging, β-carotene-linoleic acid and reducing power assays. The ethanol extract also showed better activity than that of Butylated hydroxy toluene (BHT) in the DPPH system, and showed great lipid peroxidation inhibition in the β-carotene-linoleic acid system. The essential oil showed meaningful butyrylcholinesterase activity, and α-pinene showed high acetylcholinesterase inhibitory activity while β-pinene was inactive.

The chemical constituents of the essential oil of the *Bunium persicum* Boiss seed have been studied by GC/MS technique. The major components were caryophyllene (27.81%), γ-terpinene (15.19%), and cuminyl acetate (14.67%). Individual antioxidant assays such as, DPPH scavenging activity and β-carotene bleaching have been carried out. In DPPH system, the inhibition concentration 50 (IC50) value of essential oil was determined as 0.88 mg/mL. In β-carotene bleaching antioxidant activity of essential oil (0.45%) was almost equal to BHT at 0.01%. In addition, the antioxidant activity of the oil was evaluated in crude soybean oil by monitoring peroxide and thiobarbituric acid values of the oil substrate. The results showed that the *B. persicum* essential oil (BPEO) was able to reduce the oxidation rate of the soybean oil in the accelerated condition at 60 ^{O}C (oven test). The essential oil at 0.06% showed the same effect of BHA at 0.02% (Shahsavari *et al*., 2008).

The antioxidant activity of three *Salvia* species extracts from roots and leaves in methanol have been studied, the presence of the polyphenols and tanshinones was

screened by high performance liquid chromatography (HPLC) and spectrophotometric assays and related to the antioxidant potential. The antioxidant capacity of the studied species is high, but differences between species and organs have been also revealed. *S. przewalskii* leaf extract was the strongest one in all tests, followed by *S. miltiorrhiza* root and *S. verticillata* leaf. Among the roots, the most active was *S. miltiorrhiza* extract, followed by *S. verticillata*. The antioxidant activity correlates to the total polyphenol and, depending on the assay, to the hydroxycinnamic acids content. The high content of tanshinones in both *S. miltiorrhiza* and *S. przewalskii* roots is unlikely to contribute to the antioxidant activity (Matkowski *et al*. 2008).

The *in vitro* antioxidant activities and rosmarinic acid levels of the methanol extracts of *Salvia virgata*, *Salvia staminea* and *Salvia verbenaca* was examined by Tepe (2008). The extracts were screened for their possible antioxidant activity by two complementary test systems, namely DPPH free radical scavenging and beta-carotene/linoleic acid systems. In the first case, the most active plant was *S. verbenaca*, followed by *S. virgata*. *S. staminae* exhibited the weakest antioxidant activity in this test system. In β-carotene/linoleic acid test system, *S. verbenaca* extract was superior to the other extracts studied. Antioxidant activities of BHT, ascorbic acid, curcumin and α-tocopherol were determined in parallel experiments. Activity of rosmarinic acid was also screened for better establishing the relationship between rosmarinic acid level and antioxidant activity for the plant extracts. According to the results obtained by spectrophotometric analysis and further supported by HPLC, *S. verbenaca* has the highest rosmarinic acid level. The reported results showed that the rosmarinic acid and its derivatives are more likely to be responsible for most of the observed antioxidant activities of *Salvia* species.

Antioxidant activity of essential oils of rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia officinalis* L.) was evaluated by Bozin *et al*. (2007) as a free radical scavenging capacity (RSC), together with the effect on lipid peroxidation (LP). RSC was assessed by measuring the scavenging activity of essential oils on DPPH and hydroxyl radicals. Effects on LP were evaluated following the activities of essential oils in Fe(2+)/ascorbate and Fe(2+)/H_2O_2 systems of induction. The investigated oils reduced the DPPH radical formation in a dose-dependent manner. Strong inhibition of LP in both systems of induction was especially observed for the rosemary oil.

Two new flavanone glucosides (2S)-homoeriodictyol 7,4-di-O-b -D-glucopyranoside and (2R)-eriodictyol 7,4-di-O-b -D-glucopyranoside were isolated by Yao *et al*. (2006) from the branches and leaves of *Viscum coloratum*, along with three known flavanone glucosides: (2S)-homoeriodictyol 7-O-b -D-glucopyranoside , (2S)-eriodictyol 7-O-b -D-glucopyranoside, and (2S)-naringenin 7-O-b -D-glucopyranoside. The structures of these compounds were elucidated using spectroscopic methods. The antioxidant activities of the isolated compounds were evaluated by colorimetric methods based on their scavenging effects on hydroxyl radicals and superoxide anion radicals. All the compounds showed potent albeit varied degrees of antioxidative activities.

The essential oil and methanolic extract obtained from the aerial parts of *Ziziphora clinopodioides* subsp. *rigida* (Boiss.) Rech. f. were screened by Salehi, *et al* (2005) for their possible antioxidant activity by using DPPH assay. The free radical scavenging activity of MeOH extract was superior to all other extracts, while the oil was less effective.

The antioxidant activities of the methanol extract of *Bauhinia racemosa* (MEBR) stem bark was carried out by Kumar *et al*. (2005) in various systems. DPPH radical, superoxide anion radical, nitric oxide radical, and hydroxyl radical scavenging assays were carried out to evaluate the antioxidant potential of the extract. The antioxidant activity of the methanol extract increased in a concentration-dependent manner. The MEBR inhibited the peroxidation of a linoleic acid emulsion. Similarly, the effect of MEBR on reducing power increased in a concentration-dependent manner. In DPPH radical scavenging assays the IC50 value of the extract was 152.29 μg/ ml. MEBR inhibited the nitric oxide radicals generated from sodium nitroprusside with an IC50 of 78.34 μg/ml, as opposed to 20.4 μg/ml for curcumin. MEBR also inhibited the hydroxyl radical generated by Fenton's reaction, with an IC50 value of more than 1000 μg/ml, as compared to 5 μg/ml for catechin. The amounts of total phenolic compounds were also determined and 64.7 μg pyrocatechol phenol equivalents were detected in 1 mg of MEBR.

Antimicrobial activity of medicinal plants

The plants were used in medication due to their healing potential before the discovery of the existence of microbes. There was a common belief that certain plants have potential healing features which are characterized today as antimicrobial principles (Mendonça, 2006). Although historically plants have provided a good source of antimicrobial agents in the fight against microbial infections, the use of plant derivatives as antimicrobials has been reduced by the discovery of antibiotics in the 1950's (Cowan, 1999). Due to the appearance of antibiotic resistance in the last few decades, researches were directed their effort toward the studying antimicrobial properties of plant extract, essential oils, and their constituents (Lis-Balchin and

Deans, 1997; Cowan, 1999; Sousa, *et al*. 2006). Recently alternative strategies were used including the emergence of plant derived drugs based on the isolation of plants active constituents and scientific progressing of plant extracts (Iwu, *et al*. 1999). Several scientific studies have also indicated the importance of new bioactive plant compounds against antibiotic resistant bacteria (Mendonça, 2006) some of which are pure molecules and some of them exhibit much more effective antimicrobial activities than the antibiotics (Iwu *et al*. 1999; Aburjai *et al*. 2001).

Thousands of investigations were done concerning the medicinal plants that have important inhibitory effects on all types of microorganisms in vitro (Mendonça. 2006).These investigations showed that the plants secondary metabolites have potential uses in medical procedures, pharmaceutical and food industries (Iocabellis, *et al*. 2005). Plant products are also known to possess potential for food preservation (Baratta *et al*., 1998).

Parlatan *et al* (2008) studied the structural properties of some compounds (pure chemicals from essential oils, extracts, etc.) and determined the antimicrobial activities of the fruits of Kefe cumin (*Laser trilobum* L.) obtained from different regions (Germany, India, and Turkey). The main oil components were established as limonene and perillaldehyde. The fruit extracts had a significant antimicrobial effect on pathogen bacteria such as *Staphylococcus aureus, Proteus vulgaris Proteus mirabilis*, *Bacillus cereus*, *Aeromonas hydrophila*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Enterobacter aerogenes,* and *Escherichia coli* whereas no significant antimicrobial effect of the essential oils was observed.

The essential oils of rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia officinalis* L.) was analyzed by Bozin *et al*. (2007) using GC-MS and assayed for

their antimicrobial against thirteen bacterial strains and six fungi, including *Candida albicans* and five dermatomycetes. The most important antibacterial activity of both essential oils was expressed on *Escherichia coli*, *Salmonella typhi*, *Salmonella enteritidis*, and *Shigella sonei*. A significant rate of antifungal activity, especially of essential oil of rosemary, was also exhibited.

Rojas *et al* (2006) evaluated the antimicrobial activity and minimal inhibitory concentration (MIC) of the extracts of ten medicinal plants used in Colombian folkloric medicine against five bacterial species (*Staphylococcus aureus*, *Streptococcus β-hemolític*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *E. coli*), and one yeast species (*C. albicans*). The ethanol, hexane and water extracts were obtained by standard methods. The antimicrobial activity was found by using a modified agar well diffusion method. MIC was determined in the plant extracts that showed some efficacy against the tested microorganisms. The water extracts of *Bidens pilosa* L., *Jacaranda mimosifolia* D.Don, and *Piper pulchrum* C.DC showed a higher activity against *Bacillus cereus* and *Escherichia coli* than gentamycin sulfate. Similarly, the ethanol extracts of all species were active against *Staphylococcus aureus*.

The antimicrobial activities of methanol extract of *Bauhinia racemosa* (MEBR) were determined by disc diffusion with five Gram positive, four Gram-negative and four fungal species. MEBR showed broad-spectrum antimicrobial activity against all tested microorganisms. The results obtained in the present study indicate that MEBR can be a potential source of natural antioxidant and antimicrobial agents (Kumar *et al*, 2005).

Salehi *et al*. (2005) analyzed the chemical composition of the essential oil obtained from the aerial parts of *Ziziphora clinopodioides* subsp. *rigida* (Boiss.) Rech. by GC and GC-MS. Thirty-one constituents accounting to 99.5% of the total oil were identified. Oxygenated monoterpenes (93.3%) were the predominant portion of the oil with pulegone (45.8%), piperitenone (17.4%), p-menth-3-en-8-ol (12.5%) and thymol (8.0%) as the main constituents. Antibacterial activity of the oil and its two main compounds and various extracts of plants were tested against seven Gram-(+) or Gram-(-) bacteria. It was found that the oil and methanolic extract exhibited interesting antibacterial activity.

Antimicrobial activities of the essential oil from the leaves of *Origanum syriacum* was determined by Alma *et al*. (2003) on 16 microorganisms tested using the agar-disc diffusion method, using two different concentrations of 2 and 4ml/disc, and compared with those of ampicillin, streptomycin and nystatin used as positive controls. It is evident from the results obtained that the antimicrobial (both; antibacterial and antifungal) activities greatly increase with increase of the oil concentration from 2 to 4ml/disc. It is also apparent that the oil inhibits the growth of all the bacteria and fungi except for *Kluyveromyces fragilis* and *Rhodotorula rubra*. Specifically, it is important to note that the growths of *S. aureus* and *E. coli*, two of the most common gram-positive and gram-negative food poisoning bacteria, respectively, are significantly inhibited by the essential oil at both concentrations used. The inhibition zones of the oil (at both concentrations) against *S. aureus* and *E. coli* are determined to be much higher than that of ampicillin (10mg/disc) and streptomycin (10mg/disc). On the one hand, the oil having a concentration of 4m l/disc had a remarkably higher inhibition zone in comparison to ampicillin (10mg/disc) or streptomycin (10mg/disc) for all the common bacteria studied. On the

other hand, except for *Klebsiella pneumoniae* and *Micrococcus luteus*, the oil with a concentration of 2m l/disc has a higher inhibition zone than ampicillin (10mg/disc) and streptomycin (10mg/disc).

Extraction

The most important step in assessment of the biological activity of any plant is the extraction step. Extraction may be defined as the separation of medicinally active constituents from plant parts using standard extraction procedures. The best extraction technique is the one that leads to the separation of the soluble and insoluble components and leaves behind only insoluble cellular marc. The extraction products of plants have relatively complex mixtures covering a number of groups of plant metabolites either in liquid form or semi-solid state or after removing the solvent resulting in dry powder extract. The main purpose of a standardized extraction procedure for medicinal plants is obtaining the required portion of the plant material and the elimination of unwanted material (Ics-Unido, 2006).

The efficacy of active plant constituents depend on the choice of extraction method (Shaalan, *et al*. 2005). The general techniques of extraction of medicinal plants include infusion, percolation, maceration, decoction, digestion, hot continuous extraction (soxhlet), aqueous-alcoholic extraction by fermentation, counter current extraction, phytonic extraction (with hydro-floro-carbon solvents), etc. For the aromatic plants, three types of hydro-distillation techniques steam distillation, water distillation, and combinated steam and water distillation, hydrolytic maceration followed by distillation technique and expression method may be employed (Ics-Unido 2006). New extraction methods including microwave assisted extraction, SFE, pressurized solvent extraction have been used in the last decade (Shaalan, *et al*. 2005).

In recent years, the use of microwave for extraction of constituents from plant material has shown tremendous research interest and potential. Conventional techniques for the extraction of active constituents are time and solvent consuming, thermally unsafe and the analysis of numerous constituents in plant material is limited by the extraction step (Shaalan, *et al*. 2005).

The extraction of essential oil components using solvent at high pressure or SFE has received much attention in the past several years, especially in food, pharmaceutical and cosmetic industries, because it presents an alternative for conventional processes such as organic solvent extraction and steam distillation (Xiao, *et al*. 2007). There is also a technique called enzyme assisted extraction. The mechanism for enzyme-assisted extraction is that cell wall degrading enzymes (i.e., glucanases and pectinases) can weaken or break down the cell wall rendering the intracellular materials more accessible for extraction (Li, *et al*. 2006).

Many valuable natural materials have traditionally been extracted with organic solvents. However, some of the organic solvents are believed to be toxic, and the extraction conditions are often harsh. A simple method using ethanol (a food-grade solvent) instead of methanol for the extraction of phenolic compounds is the preference frequently in the literature (Li, *et al*. 2006). The traditional techniques of solvent extraction of plant materials are mostly based on the correct choice of solvents and the use of heat or/and agitation to increase the solubility of the desired compounds and improve the mass transfer. Usually the traditional technique requires longer extraction time thus running a severe risk of thermal degradation for most of the phyto-constituents (Mandal, *et al*. 2007).

Thus the basic parameters influencing the quality of an extract are: a) the plant part used as starting material, b) the solvent used for extraction, c) the manufacturing process (extraction technology) used with the type of equipment employed, and d) crude drug: solvent ratio. The use of the appropriate extraction technology, plant material (nature of the plant material, its origin, degree of processing, moisture content, particle size), manufacturing equipment (type of extraction, filling height, hydrostatic pressure, batch size), extraction method (type of extraction, time of extraction, flow velocity, temperature and pressure) and the solvent (nature of solvent, its concentration and polarity) and good manufacturing practices, will certainly produce good desired quality of extract. From laboratory scale to pilot scale, all the conditions and parameters, if properly and accurately recorded, one can employ process simulation for successful industrial scale production (Ics-Unido. 2006).

It is nearly impossible to optimize extraction parameters for each plant material in screening studies that includes great numbers of plant species. So for screening studies generally a standardized extraction procedure is applied for all samples. Mostly ethanol and methanol in various concentrations of water are used as solvent in these studies. But ethanol is more advisable because it is much safer than methanol which has high toxic effects. The solid-liquid ratio is another important parameter in extraction of plant materials and studies indicate that mostly 1/10-50 ratios are used in screening studies.

Free Radicals

Free radicals can be defined as high energy atoms with an extra unpaired electron, that’s why they are defined to be highly reactive (Madhavi *et al.*, 1996). Oxidation is the major process that cause the formation of free radicals in living

things, drugs, and food products (Antolovich at al., 2002). Free radicals are released during the aerobic metabolism as unwanted metabolic by-products (Mantle, *et al*. 2000). They can also be produced by light energy, tobacco products, photochemical smog, alcohol, polyunsaturated fats, radiation, physical stress that leads depletion of immune system antioxidants, modification of proteins caused by gene expression changes (Pourmorad *et al*., 2006).

Due to the fact that they are unstable, free radicals have a tendency to become stabilized in a way of reducing their energy level by transferring their excess electron to other substances. As an example, when they are formed in body, they attack tissues by oxidizing cellular proteins, membrane lipids, and DNA that blocked cellular activities such as respiration and terminates the cell (Benavente-Garcia *et al*., 2000).

Antioxidative Mechanism

Antioxidant compound may, in some cases, act by multiple mechanisms depending on the reaction system due to their different characteristics (Prior, *et al*. 2005). In that manner antioxidants exhibit their antioxidant activity by various mechanisms like:; (1) chain breaking by donation of hydrogen atoms or electrons that convert free radicals in to more stable species, (2) chelating metal ions which are involved in the generation of reactive oxygen species, (3) decomposing lipid peroxides into stable final products, and (4) inhibiting the deleterious action of prooxidant enzymes (Siquet *et al*., 2006).

Antioxidants can deactivate radicals by two major mechanisms, HAT (Hydrogen Atom Transfer) and SET (Single Electron Transfer). Antioxidants with the HAT mechanism quench free radicals by hydrogen donation and with SET

mechanism antioxidants transfer one electron to reduce any compound, including metals, carbonyls, and radicals (Prior, *et al*. 2005).

Antimicrobial Agents

Antimicrobial agents are the important chemical compounds that are widely used in modern medical practice due to their disease treatment features by eliminating or killing the infecting microorganisms. There are a various number of antimicrobial agents currently available. When selecting for a particular antimicrobial agent, its selective toxicity must be evaluated. The antimicrobial agent must exhibit greater toxicity against infecting pathogens and lower against the host organism .

Antibiotics are the secondary metabolites produced by microorganisms from organic chemicals, and many antibiotics currently used are chemically modified forms of microbial biosynthetic products. Each class of antimicrobial agents represents specific interaction with a particular microorganism by exhibiting a unique mode of action (Stephen, et al. 2005). This mechanism of action depends on the type of microorganism under consideration and mainly related to the bacterial cell structure and the target sites of the microorganism (Mendonça, et al. 2006). Outermost structures of gram positive and negative bacteria possessing some main differences which affects the mechanisms of antimicrobial resistance (Holley and Patel 2005)

Modes of antimicrobial action

Antimicrobial agents have different antimicrobial activities that allow them to interfere with cell wall synthesis, inhibit protein synthesis, interfere with nucleic acid synthesis or inhibit a metabolic pathway (Stephen, et al. 2005):

1- Interference with cell wall synthesis: Some antimicrobial agents are responsible for blocking the cell wall synthesis by preventing the peptidoglycan layer synthesis.

2- Interference with the cytoplasmic membrane: These type of antimicrobial agents play role in disruption and destabilization of the cytoplasmic membrane.

3- Interference with protein synthesis by binding ribosomal subunits: Antimicrobial agents may bind to the ribosomal subunits including 30S and 50S also bind to 70S initiation complex. They cause protein chain termination and inhibition of protein synthesis.

4- Interference with DNA synthesis by blocking the enzyme DNA gyrase: Antimicrobial agents may bind to the enzyme gyrase which is responsible for winding and unwinding DNA during the replication that cause releasing of broken DNA strand into cell and leads to cell death.

Resistance to antimicrobial agents

The appearance of antibiotics resistant microorganisms is a serious problem that facing health care researchers (Cloutier 1995). The widespread and sometimes misuse of antimicrobial drugs are the most important factors that lead to the appearance of antibiotics resistant microorganisms (Lowy, 2003). The evolution of antibiotic resistance pathogenic species increased rapidly in the last sixty years (Courvalin 2005). The evolution of antibiotic resistance microbes lead to search and discover new and effective antimicrobial drugs by supplying them with mechanisms of action and new targets to work on (Cloutier 1995).

Many reasons induce the evolution of antibiotics resistant microbes, but the most important factors are the genetic mutation or transfer in structural or regulatory genes. The resistance to antibiotics may also inherited by bacteria (Courvalin 2005). The role of genetic transfer in resistance occurs by the plasmid and transposons. These plasmids are extra chromosomal circular DNA that contains a limited number of genes. Many copies of plasmids can be shared among different bacterial species (Cloutier 1995).

Plant antimicrobial agents and their mechanisms of action

Plant compounds that have antimicrobial effects can be divided into several subgroups, the most common ones are represented below.

1- Flavonoids: They are accepted to be the effected antimicrobials against a wide range of microorganisms. Their activity is thought to interact with extracellular and soluble proteins also for ability to form complex with bacterial cell walls.

2- Simple phenols and phenolic acids: These are the simplest bioactive plant compounds. Their toxicity to microorganisms is associated with the site(s) and number of hydroxyl groups they have. The main mechanism of action of phenolic compounds thought to be related with their toxicity to microorganisms that cause enzyme inhibition through a reaction between oxidized compounds and sulfhydryl groups or by other nonspecific interractions of the proteins and oxidized compounds.

3- Quinones: They are an important group of plant compounds that may form irreversible complex with nucleophilic amino acids in proteins. This action leads to inactivation of proteins and loss of function. Their mechanisms of action associate

with some targets in the microbial cell including the cell wall polypeptides, surface-exposed adhesins and membrane bound enzymes.

4- Terpenoids and essential oils: These are the secondary metabolites consist of isoprene structure and called terpenes. Their mode of antimicrobial action is not fully understood but they are thought to be involved in membrane disruption by the lipophilic compounds (Cowan, 1999). Many of the terpenes exhibit antimicrobial action on a wide range of microorganisms including the gram negative and positive bacteria. They are generally known with their toxic effects on membrane structure and function of microorganisms owing to their lipophilic character that results in membrane expansion, increased membrane fluidity and permeability, disturbance of membrane proteins, inhibition of respiration, and alteration of ion transport process (Trombetta, *et al*. 2005).

5- Tannins: They contain two subgroups as hydrolyzable and condensed tannins. Their ability to inactivate microbial adhesins, enzymes cell envelope transport proteins is thought to from their mode of antimicrobial action.

6- Alkaloids: They are the phytochemicals that composed of heterocyclic nitrogen compounds. Morphine is a quite important compound as the first medically useful example of an alkaloid which was isolated in 1805 from the opium popy *Papaver samniforum* (Balunas and Kinghorn 2005). Alkaloids have mechanism of action particularly with the ones have highly aromatic planar quaternary structures, such as berberine and harmane is attributed to their ability to intercalate with DNA (Cowan, 1999).

Antimicrobial evaluation technique

The screening methods for antimicrobial susceptibility test are of great important in order to detect plant antimicrobial activity. Three screening method groups currently available for detection of antimicrobial activity of natural products, these methods include bioautographic, diffusion, and dilution method. The bioautographic and diffusion methods can be described as qualitative techniques by which only detect presence or absence of substances with antimicrobial activity. On other hand dilution methods are known as quantitative test since they determine the MIC (Valgas, *et al*. 2007).

There has been much research interest in agar-based testing of disc-diffusion method, and commonly used owing to its relative simplicity and the lack of need for specialized equipment (Scorzoni, *et al*. 2005). Antimicrobial susceptibility discs are absorbent, paper discs that are impregnated with a specific antimicrobial agent and are usually marked with the drug concentration and the disk code. The test procedure is based on the Kirby-Bauer method. Discs are placed on the surface of an agar plate that is inoculated with the organism to be tested then incubated overnight. Clinically the in vitro antimicrobial susceptibility is used to determine antimicrobial agent of choice whenever the susceptibility of a bacterial pathogen is unpredictable (Hansen, *et al*. 1996). The liquid-dilution method also allows detecting of whether a compound or extract has antimicrobial action at a particular concentration. The serial dilution test was reported to give the most reproducible results on the MIC and was recommended as general standard methodology for the testing of natural products (Burrows, *et al*. 1993, Hansen, *et al*. 1996). The MIC generally is considered the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. It is defined as the lowest concentration of an antimicrobial agent that is required to inhibit the growth of a particular organism in a well-standardized in vitro susceptibility test.

The conventional technique for measuring the MIC involves exposing the test organism to a series of two-fold dilutions of the antimicrobial agent in a suitable culture media (e.g. broth or agar formats) (Jorgensen *et al*. 2004). MIC information is quantitative rather than qualitative, it may be useful for dosage determinations as well as antibiotic selection (Burrows, *et al*. 1993). Determinations of MIC are influenced by numerous variables, including the composition of the test medium, the size of the bacterial inoculum, and the duration of incubation. Also the in vitro test conditions cannot encompass all factors that can have an influence on in-vivo antimicrobial activity (Burrows, *et al*.1993).

Botanical Description of Studied Plant Species

Achillea fragrantissima Forssk. (*Asteraceae*) (Yarrow القيصوم)

Fragrant charnaephyte. 50-100 cm, white-woolly, many~stemmed from a woody base, paniculate-corymbose above. Stems virgate, rigid. Leaves small, oblong or ovate, undivided, thickish, canescent, later glabrescent, callous-serrulate. Heads discoid, in lax corymbs. Involucre 5 mm, oblong-ovoid, canescent. Flowering: March-October (also as late as December-January). Habitat: Wadi beds in deserts. Common. Lower Jordan Valley, Dead Sea area, Arava Valley, Amman, Mafraq. Area: E. Sahara-Arabian and W. Irano-Turanian. (Post, 1932; Zohary and Feinburn-Dothan, 1986)

Achillea millefolium L. (*Asteraceae*) (Yarrow القيصوم)

Erect herbaceous perennial plant that produces one to several stems (0.2 to 1m tall) and has a rhizomatous growth form. Leaves are evenly distributed along the stem, with the leaves near the middle and bottom of the stem being the largest. The leaves have varying degrees of hairiness (pubescence). The leaves are 5–20 cm long, bipinnate or tripinnate, almost feathery, and arranged spirally on the

stems. The leaves are cauline and more or less clasping. The inflorescence has 4 to 9 phyllaries and contains ray and disk flowers which are white to pink. There are generally 3 to 8 ray flowers that are ovate to round. Disk flowers range from 15 to 40. The inflorescence is produced in a flat-topped cluster. Flowering: May-June, and is a frequent component in butterfly gardens. Common yarrow is frequently found in the mildly disturbed soil of grasslands and open forests. Active growth occurs in the spring. (Post, 1932; Zohary and Feinburn-Dothan, 1986)

Juniperus phoenicea L. (*Cupressaceae)* (Juniper العرعر)

Large shrub or small tree reaching 2–12 metres (6.6–39 ft) tall, with a trunk up to 1 metre (3.3 ft) diameter and a rounded or irregular crown. The leaves are of two forms, juvenile needle-like leaves 8-10 mm long on seedlings, and adult scale-leaves 0.5-2 mm long on older plants; they are arranged in opposite decussate pairs or whorls of three. It is largely monoecious, but some individual plants are dioecious. The cones are berry-like, 6-14 mm in diameter, orange-brown, occasionally with a pinkish waxy bloom, and contain 3-8 seeds; they are mature in about 18 months. The male cones are 2-4 mm long, and shed their pollen in early spring. Habitat: Common inTafila and Dana. (Post, 1932; Zohary and Feinburn-Dothan, 1986)

Lavandula dentata **(***Lamiaceae*) (Lavender لافندر)

This is an aromatic shrub grows up to nearly a meter in height. The gray-green, linear or lance-shaped leaves have toothed edges and a lightly woolly texture. Its native habitat includes low hills with limestone substrates amidst other shrubs. It is present on Madeira and the Canary Islands. (Post, 1932; Zohary and Feinburn-Dothan, 1986)

Lavenders (*Lavandula dentata*) of the genus *Lavandula* are extensively found in the Mediterranean region and are often cultivated in Spain, France and Italy (Goren *et al*., 2002) and Jordan. Lavenders, either as an essential oil or in dried form have been used since centuries for many cosmetic and therapeutic purposes. Its medicinal importance is evident by extensive literature (Poucher, 1974). Some of the drugs derived from this plant are part of many Pharmacopeia (Saaed, 1970). This plant is used as carminative, antispasmodic, deobstruent, stimulant, expectorant, and resoluent. The essential oil extracted from it has long been used as a medication against chest and colic affections, to relieve biliousness, nervous headache and also for cleansing wounds (Hussain *et al.*, 1988). This essential oil is also supposed to be carminative (smooth muscle relaxing), antifungal, antibacterial, antidepressive, sedative and effective for insect bites and burns (Shellie *et al*., 2002).

Mentha aquatica L. **(***Lamiaceae*) (Menth النعناع)

Perennial herb, 30-100 em, often canescent, more or less villose, sometimes glabrescent. Stems more or less erect, simple or branched. Leaves petiolate, ovate to nearly orbicular, rounded or subcordate at base, serrate, usually canescent on both faces. Inflorescence of 1-3(-4) verticillasters crowded into a terminal head about 2 em in diameter, generally exceeded by floral leaves, sometimes with 1-2 additional verticillasters below the terminal one; bracteoles lanceolate. Calyx 3.5-4 mm, tubular, sulcate, pubescent and with yellow sessile glands; throat naked; teeth nearly equal, triangular-subulate. corolla purplish-pink. Flowering: July-November. Habitat: In streams and rivulets. Common Upper Jordan Valley and Irbid. Area: Borealo- Trop. (Post, 1932; Zohary and Feinburn-Dothan, 1986)

The genus *Mentha* L., consisting of more than 25-30 species grows widely throughout temperate regions of the world (Gulluce *et al*., 2007). *Mentha arvensis*

(*M.arvensis*), *Mentha piperita* (*M. piperita*), *Mentha longifolia* (*M longifolia*) and *Mentha spicata* (*M. spicata*), commonly known as menthol mint, peppermint, wild mint and spearmint, respectively are frequently cultivated in several countries of East Asia, Europe, America and Australia for the production of essential oils (Gulluce *et al*., 2007). The essential oils from *Mentha* species have been in use by human being since ancient times (Iscan *et al*., 2002). The aerial parts of *Mentha* species are commonly used in commercial spice mixtures for many processed foods as well as in herbal teas (Moreno *et al*., 2002; Kofidis *et al.*, 2006). Moreover, the essential oils from *Mentha* species are generally employed to flavour liqueurs, breads, salads, soups and cheese, as well as an ingredient of cosmetics (Yadegarinia *et al*., 2006). In addition these have been utilized traditionally for the treatment of many digestive tract diseases due to its carminative, antiemetic spasmodic, analgesic, and anti-inflammatory attributes (Moreno *et al*., 2002; Gulluce *et al*., 2007). The essential oils of some *Mentha* species including *M. arvensis*, *M. piperita*, *M. longifolia* and *M. spicata* are potential candidates for exhibiting antimicrobial, antioxidant and radical-scavenging activities (Dorman *et al*., 2003; Gulluce *et al*., 2007), which are mainly attributed to the presence of phenolic substances (Hosseinimehr *et al*., 2007).

Origanum syriaca (L.) Rafin. (*Lamiaceae*) (Oregano الزعتر)

Chamaephyte, 30-50 cm, woolly-canescent and glandular. Stems erect, rigid paniculately branched, ending in spike-like inflorescences. Leaves short-petiolate to sessile, entire, ovate, obtuse, rather thick, with elevated veins on lower face; floral leaves obovate, as long as calyx. Spikes oblong, 1-2 cm; cymes short-pedunculate. Calyx 2-2.5 mm, slit on outer side, not dentate. Corolla white, 4 mm; tube exserted. Flowering : April- September. Habitat: Batha on rockey hills; also on walls. Common. Amman, upper Jordan valley and Irbid. Area: Mediterranean. (Post, 1932; Zohary

and Feinburn-Dothan, 1986)

The genus *Origanum* (oregano) is significant in the family Lamiaceae , perennial and shrubby herbs, widespread throughout the world (Kordali *et al*., 2008). The genus includes some important culinary herbs, including sweet marjoram (*Origanum syriaca* L), commercially available and exportable plants with appreciable market values (Esen *et al*., 2007). *Origanum* plant are extensively used for the flavoring of alcoholic beverages, food products and in perfumery due to their spicy fragrance (Olivier, 1994; Filippo-D-Antuono *et al*., 2000). Besides their commercial importance, such plants have been used, for long, as condiments and spices for foods like salads, soups, sausages and meats (Baydar *et al*., 2004). Their use for the treatment of various diseases was also in practice, being sudorific, expectorant, stomachic, antiseptic, and stimulant (Ozcan, 1998). Both academia and the food industry have been interested in the biological properties of *Origanum* extracts and essential oils due to their antimicrobial and antioxidant potential (Sagdic and Ozcan, 2004).

Rosmarinus officinalis L. (*Lamiaceae*) (Rosmarry اكليل الجبل)

One or more, branched erect densely leavy stem. Leaves persistent, coriaceous, linear 1 – 2.5 cm long, revolute-margine, olive green at upper surface, canescent at lower. Flowrs sessile, opposite, arranged in axillary racems 2-3 cm long; bracts minutes, oblong to ovate, caducous; calyx pubescent-canescent; corolla twice and a half long as calyx. Flowering: all the year. (Post, 1932; Zohary and Feinburn-Dothan, 1986)

Rosmarinus officinalis L. (Rosemary) is a very important medicinal and aromatic plant, which belongs to the genus *Rosmarinus* of the Lamiaceae family. Rosemary, a perennial herb, has fragrant evergreen needle-like leaves (Bousbia *et al*.,

2009). Anthropologists and archaeologists have found evidence that rosemary herbs were widely used in folk medicine, culinary and cosmetic virtues in the ancient Egypt, Mesopotamia, China, India and Pakistan and for the flavouring of food products (Pintore *et al*., 2002). *Rosmarinus officinalis* essential oil is of immense medicinal worth for its powerful antimutagenic, antiphlogistic, antioxidant, chemopreventive and antibacterial properties (Koschier and Sedy, 2003; Oluwatuyi *et al*., 2004; Celiktas *et al*., 2007).

Salvia palaestina Benth. (*Lamiaceae*) (Sage خرنة)

Perennial herb, 30-80 cm, strigose; stem ending in a panicle 20-40 cm broad. Leaves rugose, greenish to canescent, the lower long-petioled, oblong 10-20 long, often pinnatisect or lyarate at base of blad, the lateral segment small, confluent, and the terminal 8-10 cm long, 2-3 cm broad, crenate, often lobed or incised. Floral leaves membranous, often colored, sparingly papillose-pubescent, orbicular to ovate, cuspidate, shorter than galyx; galyx sparingly papillose 1.5 cm long, growing in fruit, bilabiate, with lanceolate, prickely-mucronate teeth; corolla white, twice to thrice as long as galyx, the upper lip rather straight. Flowering: April- June. (Post, 1932; Zohary and Feinburn-Dothan, 1986), Habitat: Fields and hills. Common Madab, irbid and Moaab. Area: W. Irano-Turanian, extending into the E. Mediterranean.

In the Lamiaceae family, *Salvia* is the biggest genus which consists of about 900 species. The *Salvia palaestina,* one of the common species of this genus is popularly used as a culinary herb for flavoring and seasoning. *Salvia palaestina* has a variety of medicinal uses such as astringent, antiseptic and spasmolytic (Ahmadi and Mirza, 1999). Salvia species have been employed locally as traditional medicine to treat a variety of diseases such as wounds, malaria, microbial infections and cancer (Kamatou *et al*., 2008). Salvia species also exhibited various in-vitro pharmacological

properties (Kamatou *et al*., 2006). Various phenolic compounds in plants related to this genus have shown excellent antioxidant capacity as well as antimicrobial activity (Tepe *et al*., 2005).

Thymus capitatus (L.) Hoffmanns. & Link **(***Lamiaceae*) (Thyme زعتر)

Chamaephyte, 20-40 cm. Roots densely and finely ramified, penetrating between rock layers. Stems white-tomentose, erect or ascending, with numerous rigid branch lets bearing short, densely leafy brachyblasts; previous years' branchlets spinescent. Leaves rigid, linear, subacute, 2-8 mm, keeled and boat-shaped, glandular-punctate and sparsely ciliate. Heads ovoid, dense, 1-1.5 cm; floral leaves densely imbricate; bracteoles about as long as floral leaves, flat and thinner in texture, oblanceolate, ciliate, concealing the calyx. Flowers nearly sessile. Calyx 4-5 mm; teeth ciliate; those of the upper lip connivent. Corolla purplish-pink, pubescent and glandular-punctate. Flowering: May-October. (Post, 1932; Zohary and Feinburn-Dothan, 1986)

Hab.: Batha on rocky ground and compact soil. Very common and abundant in Amman, Area: Mediterranean.

The genus *Thymus* comprising of around 350 species of perennial, aromatic herbs and subshrubs is predominantly found in Mediterranean region, Asia, Southern Europe and North Africa (Maksimovic *et al*., 2008). Thymus species are considered as medicinal plants due to their pharmacological and biological properties. In native medicine, flowering parts and leaves of *Thymus* species have been extensively used as herbal tea, tonic, carminative, antitussive and antiseptic, as well as for treating colds (Rota *et al*., 2004; Maksimovic *et al*., 2008; Rota *et al*., 2008)

Varthemia iphinoides Boiss. & Bl. *(Asteraceae*) (Varthemia كتيلة)

Chamaephyte, 40-60 cm. Stem numerous, erect, vigate, glandular-tomentose and villose, each ending in a long and narrow, short-branched inflorescence. Leaves densely silky, oblong, obtuse, half-clasping. Involucre 8-100 mm; involucral bracts loosly imbricate, silky- tomentose, oblong, obtuse; outer bracts herbaceous at tip; inner bracts membranous. Pappus reddish, about twice as long as achene.Flowrning: September – November. Habitat: Rock outcrops. Common. Amman, Jarash, and Rehab. Area: Mediterranean. (Post, 1932; Zohary and Feinburn-Dothan, 1986)

CHAPTER TWO

MATERIALS AND METHODS

Plant Materials

The aerial parts of plant species were collected during spring and summer (2009-2011) and taxonomically identified by Prof. Sawsan Oran (department of biological sciences, Jordan University). Voucher specimens of collected plants were deposited at Jordan university herbarium. The collected plants are shown in the photos and table below:

Plant Species	Family	Common name	Site of collection
Achillea fragrantissmia Forssk	*Asteraceae*	Yarrow	Mafraq
Achillea millefolium L.	*Asteraceae*	Yarrow	Ramtha and Rehab
Juniperus phoenica L.	*Cupressaceae*	Juniper	Tafila (Dana)
Lavandulla dentata	*Lamiaceae*	Lavander	Mafraq
Mentha aquatica L.	*Lamiaceae*	Menth	Irbid (Um Qais)
*Origanum syriac*a (L.) Rafin.	*Lamiaceae*	Origano	Irbid
Rosmarinus officinalis L.	*Lamiaceae*	Rosemary	Mafraq
Slavia palaestina Benth.	*Lamiaceae*	Sage	Rehab
Thymus capitatus (L.) Hoffmanns. & Link	*Lamiaceae*	Thyme	Amman (Abdon)
Varthemia iphinoides Boiss. & Blanche	*Asteraceae*	Varthemia	Amman and Rehab



Achillea fragrantissmia



Achillea millefolium



Juniperus phoenica



Lavandulla dentata



Mentha aquatica



*Origanum syriac*a



Rosmarinus officinalis



Slavia palaestina



Thymus capitatus

Varthemia iphinoides

Preparation of plant extracts

The plant extracts was prepared using the modified method of Alade & Irobi (1993). Ten gram portions of the dried powdered plant were soaked separately in 100 ml of methanol, ethanol (98 %) and ether, for 24h with agitation at 150 rpm. Then, the extracts were filtrated. The obtained filtrates were concentrated under vacuum at 40°C using rotary evaporator to obtain the dry extracts. The extracts were reconstituted using dimethyl sulphoxide (DMSO) to get 200 µg/ml concentrations.

Extraction of essential oils

The essential oils of the plants were extracted using steam distillation method. The fresh aerial parts of collected plants were submitted for 3 h to steam - distillation using a stem distillation apparatus deigned in the lab. The obtained essential oil was dried over anhydrous sodium sulphate and stored at +4 °C until tested and analyzed. The yield oil yield of each plant was recorded.

Determination of antimicrobial activity

Microorganisms used

The microorganisms that were used in this study are mentioned in the table below:

Bacteria species	**Fungal species**
Staphylococcus aureus ATCC 6538	*Candida albicans* ATCC 10231
Bacillus subtilis ATCC 11780	*Aspergilllus niger* IMI 309921
Pseudomonas aeruginosa ATCC 9027	*Mucuor mehei* IMI 125823
Escherichia coli ATCC 25922	*Trichophyton mentagrophytes* ATCC 9538
	Fusarium oxysporum

Agar diffusion assay

The modified agar well diffusion method of Perez *et al.*, (1990) was implemented. Each medium was inoculated with the suspended microorganism. Once the agar solidified, it was punched with a six millimeters diameter wells and filled with 100 μL of the plant extracts or essential oils (20% v/v in DMSO) and blanks (ethanol, methanol, and ether). Simultaneously, gentamycin sulfate (*P. aeruginosa*,and *E. coli*), penicillin G (*S. aureus*, and *B. cereus*), floconazole and nystatin (Fungal species) was used as positive controls at a concentration of 200 μg/ml. The plates then incubated at 37 °C for 24 h for bacteria, and 27 °C for 72 – 96 hr for fungi. The inhibition zones were measured and recorded. The antimicrobial activity was calculated by applying the expression:

$$\% \text{ RIZD} = ((\text{IZD sample} - \text{IZD negative control})/ \text{ IZD antibiotic standard}) \times 100 \%$$

(Perez *et al.*, 1990)

Where RIZD is the percentage of relative inhibition zone diameter and IZD is the inhibition zone diameter (mm). Equation compensates the possible effect of the solvent (blank) other than water on the IZD. The resulting IZD of the samples would be either higher or equal to the IZD of the blanks. Therefore, the obtained percentages is positive. The test was considered negative (-) when the IZD of the sample equal to the IZD of the blank.

Minimal inhibitory concentration (MIC) evaluation

The MICs were determined using 96-well microtitre plates. The bacterial or spore suspension suspension was adjusted with sterile saline to a concentration of 1.0 x 10^5 (colony forming unit) CFU/ml (spore/ml for fungi). Essential oil to be

investigated were added to broth medium (100 μl) with microorganism inoculums (1.0×10^4 CFU per well for bacteria and 1.0×10^4 spore for fungi) to achieve the wanted concentrations (2.5-1500 μg/ml). The microplates were incubated for 24 h at 37°C for bacteria and 72- 96h at 27°C. The lowest concentrations without visible growth (at the binocular microscope) were defined as concentrations that completely inhibited bacterial growth (MICs).

Evaluation of the effect of the essential oils on the spore germination of the tested fungi

Antifungal activity of essential oils on spore germination was tested using slide technique (Nair and Ellingboe , 1962) by mixing the plant essential oils with spore suspension on a dried clean slide then slide was placed on glass rod in Petri-dish under moisten conditions and incubated for 24h at 27± 1°C. Four microscopic fields (x = 10 x 40) for each replicated (slide) was used. The percentage of spore germination was calculated according to the following formula:

$$\text{Spores germination (\%)} = (\text{Spores germination (no.)} / \text{Total spores (no.)}) \times 100$$

Evaluation of the mode of action of plant essential oils on microorganisms

The mode of action of plant essential oils on the microorganisms was evaluated by the method of Haraguchi *et al.* (1996). A mixture of fatty acids (palmatic acid, oleic acid and linolic acid) at concentration 14μg/ml was added to liquid media (Nutrient broth for bacteria and Malt extract for fungi) the media were inoculated with bacteria or fungi and the cultures then incubated at 37 °C for 24 h for bacteria, and 27 °C for 72 – 96 hr for fungi, the growth was compared with cultures that contain the essential oils at MICs concentration recorded from previous test. The results were compared to control cultures.

To evaluate the effect of addition of amino acids, casamino acid was added at concentration 20 µg/ml at the same method. A mixture of nucleotides at concentration 100 µg/ml was added to evaluate the effect nucleotide addition (Haraguchi *et al.* 1996).

Determination of antioxidant Activity

The antioxidant activity of the plant extracts and essential oils were determined using the following tests:

DPPH radical scavenging activity

The free radical scavenging capacity of the essential oils was determined using DPPH. A methanol DPPH solution (0.15%) was mixed with serial dilutions (2.5µg to 160 µg) of plant essential oils and after 10 min, the absorbance was read at 515 nm using a spectrophotometer. Vitamin C used as a standard. The inhibition curve was plotted (Viturro *et al*. 1999). The DPPH radical scavenging was calculated as mentioned below.

$$\%\ \text{DPPH radical scavenging} = \frac{\text{Acontrol} - \text{Atest}}{\text{Acontrol}} \times 100$$

Nitric oxide radical inhibition assay

Nitric oxide radical inhibition can be estimated by the use of Griess Illosvoy reaction (Garrat, 1964). In this investigation, Griess Illosvoy reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-napthylamine (5%). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and plant essential oils (5 µg to 160 µg) or standard solution (rutin, 0.5 ml) was incubated at 25 Cº for 150 min. After incubation, a 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) was

added to 0.5 ml of the reaction mixture and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride added, mixed and allowed to stand for 30 min at 25 Cº. The formed pink coloured chromophore was quantified using 540 nm absorbance against corresponding blank solutions. The scavenging % was calculated as below.

$$\text{Nitric Oxide scavenged (\%)} = \frac{\text{Acontrol - Atest}}{\text{Acontrol}} \times 100$$

Where, Acontrol = Absorbance of control reaction and Atest = Absorbance in the presence of the essential oils.

Superoxide anion scavenging activity

Measurement of superoxide anion scavenging activity of plant essential oils was done based on the Nishimiki method (1972). About 1 ml of nitroblue tetrazolium (NBT) solution (156 µM NBT in 100 mM phosphate buffer, pH 7.4) 1 ml NADH solution (468 µM in 100 mM phosphate buffer, pH 7.4) and 0.1 ml of sample solution of plant essential oils (1.25 µg to 10 µg) in water mixed. The reaction was initiated by adding 100 µl of phenazine methosulphate (PMS) solution (60 µM PMS in 100 mM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25Cº for 5 min, and the absorbance at 560 nm was measured against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. Curcumin was used as a positive control and scavenging % calculated as mentioned above.

Lipid peroxidation assay

The rat liver microsomal fraction was prepared by the method of Bouchet *et al*(1998). The reaction mixture contained in a final volume of 1.0 ml, 500 µl of liver microsomal fraction, 300µl buffer containing the plant essential oils (10-150µg), 100

μl of $FeCl_3$ (1 mM) and 100μl ascorbic acid (1 mM) to start peroxidation. Samples was incubated at 37°C for 1 hour, after that lipid peroxidation was measured using the reaction with thiobarbituric acid (TBA). Thiobarbituric acid reactive substances was determined by the methods of Houghton *et al*. (1995) and Aruoma *et al* (1989). The absorbance of the organic layer was measured at 532 nm. Vitamin E was used as a standard.

Hydroxyl radical scavenging assay

The assay was made as described by Halliwell method (1987) with minor changes. All solutions were prepared freshly. 1.0 ml of the reaction mixture contained 100 μl of 28 mM 2-deoxy-2-ribose (dissolved in phosphate buffer, pH 7.4), 500μl solution of various concentrations of the plant (10 to 80 μg), 200μl of 200μM $FeCl_3$ and 1.04 mM EDTA (1:1 v/v), 100 μl H_2O_2 (1.0 mM) and 100μl ascorbic acid (1.0 mM). After an incubation period of 1 hour at 37 C^o the extent of deoxyribose degradation was spectrophotometrically measured by the TBA reaction using 532 nm wave length. Vitamin E will be used as a positive control.

Reducing power

The reducing power of plant extract will be determined according to the Oyaizu method (1986). Different concentrations of plant essential oils (2.5 μg – 160 μg) were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50C^o for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) added to the mixture, which then was centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml. 0.1%) and the absorbance measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Butylated hydroxy toluene (BHT) used as a standard.

Determination of the chemical constituent of the essential oils using Gas chromatography-Mass spectrometry (GC-MS) analysis

The chemical compound and the ratio of each in the essential oils was determined by using GC-MS. Varian chrompack CP-3800 GC/MS/MS-200 equipped with split-splitless injector and DB-5 GC column (5% diphenyl 95% dimethyl polysiloxane), (30 m × 0.25 mm ID, 0.25 μm film thickness) was used. The injector temperature was set at 250C^o with a split ratio of 1:10. Detector and transfer-line temperatures were 160C^o and 230 C^o respectively. A linear temperature program was used to separate the different oil components. Temperature programming was applied at 3°C/min heating rate starting from 60 C^o (initial temperature) to 250 C^o (final temperature) and held at 250C^o for 5 min with a total run time of 60 min. Each sample was analyzed twice. The mass detector was set to scan ions between 40-400 m/z using full scan mode and electron impact (EI, 70 eV). A hydrocarbon mixture of n-alkanes (C8-C20) was analyzed separately by GC-MS using the same column (DB-5) and under the same chromatographic conditions. Linear retention index (Kovat's index) was calculated for each component (each peak) separated by GC-MS using the values of its retention time and the retention times of the reference n-alkanes applying the Van den Dool equation (Van Del Dool & Kartz, 1963). Then identification of the components of the oil was performed by matching their spectra with the data bank mass spectra (WILEY, NIST and ADAMS-2007 libraries) and also by comparing their linear retention indices (Kovat's indices) with reported values in literature, mainly the Adam's library (Adams, 2007). Identification of certain compounds (e.g. α-pinene, β-pinene, myrcene, δ-2-carene, limonene, 1,8-cineole, Z-β-ocimene, E-β-ocimene, linalool, camphor, borneol, isobornyl acetate, β-caryophyllene, thymol, carvacrol, and heptadecane) was further confirmed by co-chromatography of their

authentic standards (purchased from Sigma-Aldrich) under the same chromatographic conditions mentioned above.

The oils steam distilled from plants were analyzed and the relative peak area for each component of the oil was measured. Concentrations of oil components were calculated as % content using their relative peak areas assuming a unity response by all components. Each sample was analyzed twice.

Statistical analysis

All the expermints were performed four time ($n = 4$), the statistical analysis was done using SPSS package 15. The results were analyzed using one way ANOVA test and paired mean test at ($P < 0.05$).

CHAPTER THREE

RESULTS

Antimicrobial activity of plant extract and essential oils

Antibacterial activity

The antibacterial activities of plant extracts and oils are given in figures (1 – 4). These results show that the oils of all tested plant species possess significant antibacterial activity as compared to the control (standard antibiotic). The plant extracts obtained using different solvents (ethanol, methanol and ether) posses lower activity than the essential oil and variable RIZD comparing to standard antibiotic.

All the used medicinal plants essential oils and the various extracts possess antibacterial activity against *S. aureus* (Figure 1). The oil of *V. iphinoides* shows the highest antibacterial activity with RIZD = 281.48 ± 12.47 %, while the essential oil of *L. dentata* shows the lowest activity with RIZD 79.63 ± 7.71%. The methanolic extracts of most plants gave lower activity against *S. aureus* compared with the oils and higher activity than the extract obtained by ethanol and ether.

The oil of *M. aquatia* possess significant antibacterial activity against *E. coli* (Figure 2) with RIZD = 188.64 ± 8.7 %, and the lowest RIZD was 89.77 ± 7.76 % for *L. dentata* essential oil. All other plant extracts show lower activity against *E. coli* compared to the oils. The extracts obtained using methanol gave higher antibacterial activity against *E. coli* than the extract obtained using other solvents.

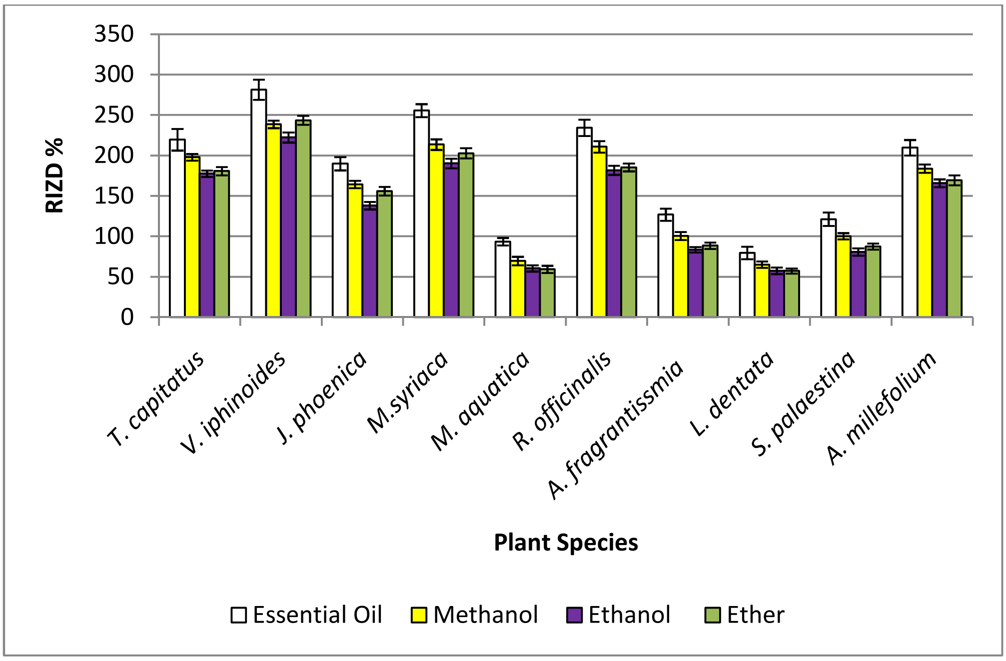


Figure 1: Antibacterial activity of medicinal plant extracts and essential oils against *S. aureus*.

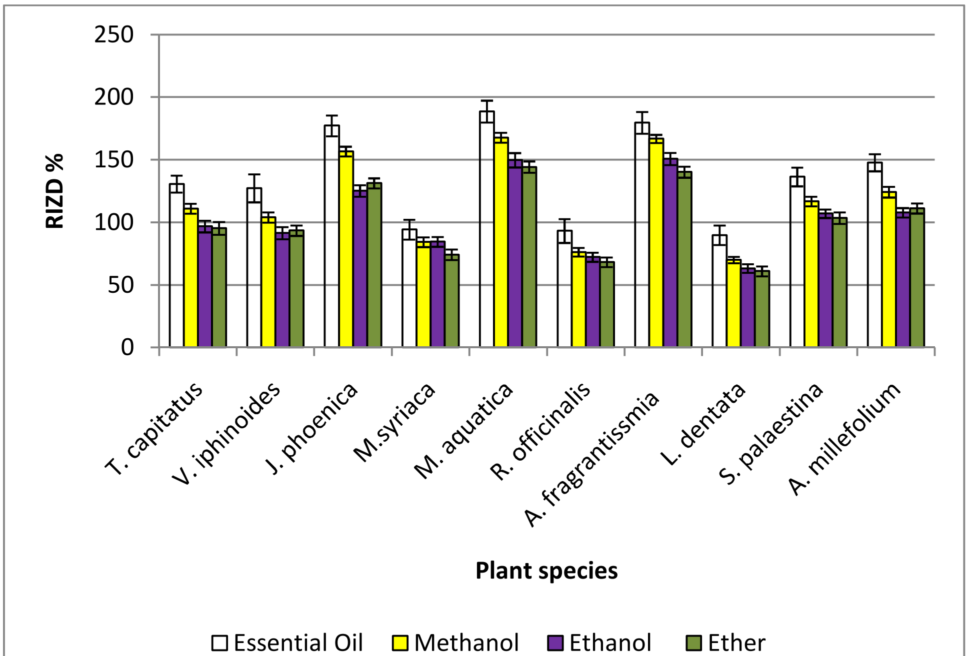


Figure 2: Antibacterial activity of medicinal plants extract and essential oils against E. *coli*

The antibacterial activities of the plant extracts and oils against *B. Subtilis* are shown in figure (3). The oils of seven plants possess higher activity compared to standard antibiotic, the RIZD for these oils range between 100.96 ± 4.84 % to 169.23 ± 11.32 %. While the oils of other plants (*O. syriaca, L. dentata* and *R. officinalis*) possess lower activity than standard antibiotic with RIZD between 80.77± 9.42 %and 92.31 ± 5.44 %. All extracts obtained by different solvents showed antibacterial activity against *B. subtilis* with the highest activity for methanolic extract.

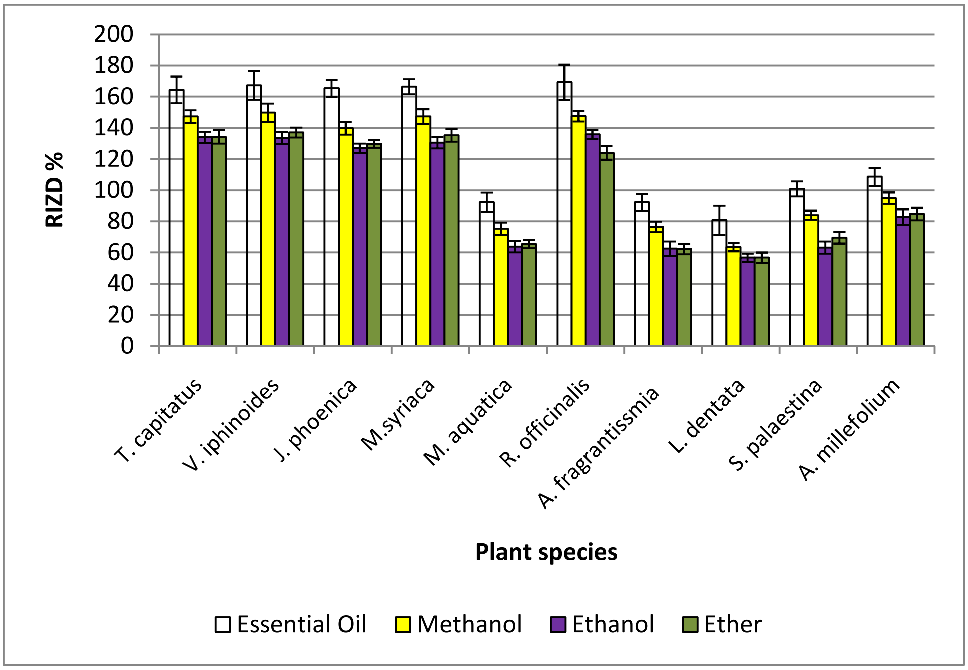


Figure 3: Antibacterial activity of medicinal plant extracts and essential oils against *B. subtilis*

The oils of *V. iphinoides* and *J. Phoenica* (Figure 4) showed significant antibacterial activity against *P. auregenosa* compared to other oils, the RIZD for these two oils were 140.18 ± 6.1 % and 135.71 ± 5.05 % respectively. The oil that possessed the lowest antibacterial activity against *P. auregenosa* was the *L. dentata* essential oil that have 61.61 ± 3.42 % RIZD. The other oils were shown to be within the same previous RIZD ranges. The mathanolic, ethanolic and ether plant extracts possess less antibacterial activity than the oils.The methanolic extract of *V. iphinoides* and *J. phoenica* showed significant RIZD (127.5 ± 2.89 % and 126 ± 3.16 %, respectivily) as compared to other plant extracts.

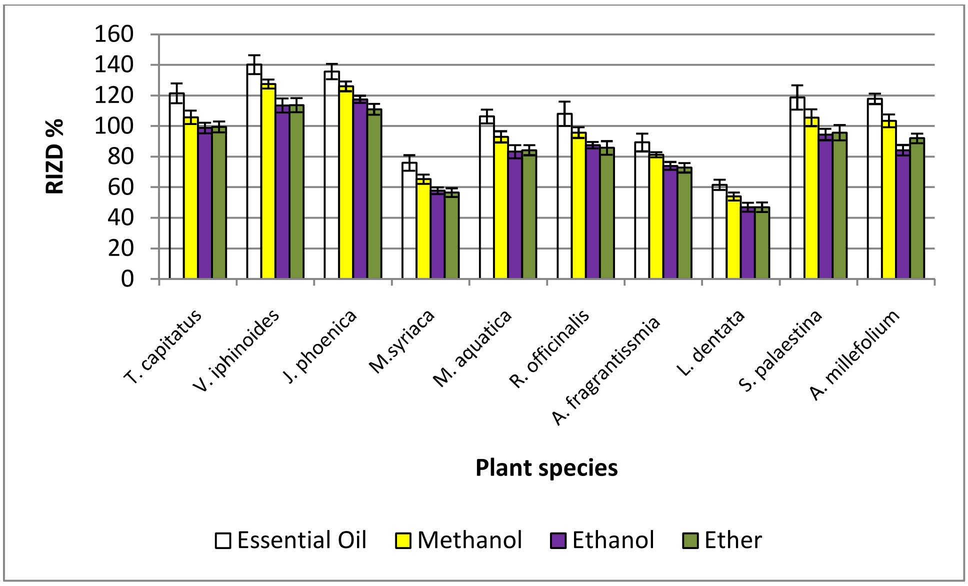


Figure 4: Antibacterial activity of medicinal plant extracts and essential oils against *P. auregenosa*.

Antifungal activity

The antifungal activities of plant extracts and essential oils are given in figures (5–9). The results show that the oils of all tested plant species possessed significant antifungal activity as compared to that of standard antifungal agent. The plant extracts obtained using different solvents (ethanol, methanol and ether) possessed lower activity than the oil and variable RIZD compared to standard antifungal agent.

The antifungal activities of plant extracts and essential oils against *C. albicans* are shown in figure (5). The oils of *V. iphinoides* and *A. millefollium* showed 168.48 ± 9.64 % and 157.61 ± 5.47% RIZD compared to standard antifungal, while the essential oil of *L. dentata* showed the lowest RIZD (85.85 ± 7.43). The methanolic extract of *V. iphinoides* showed the significant RIZD (152.75 ± 5.19 %) compared to other plant extract.

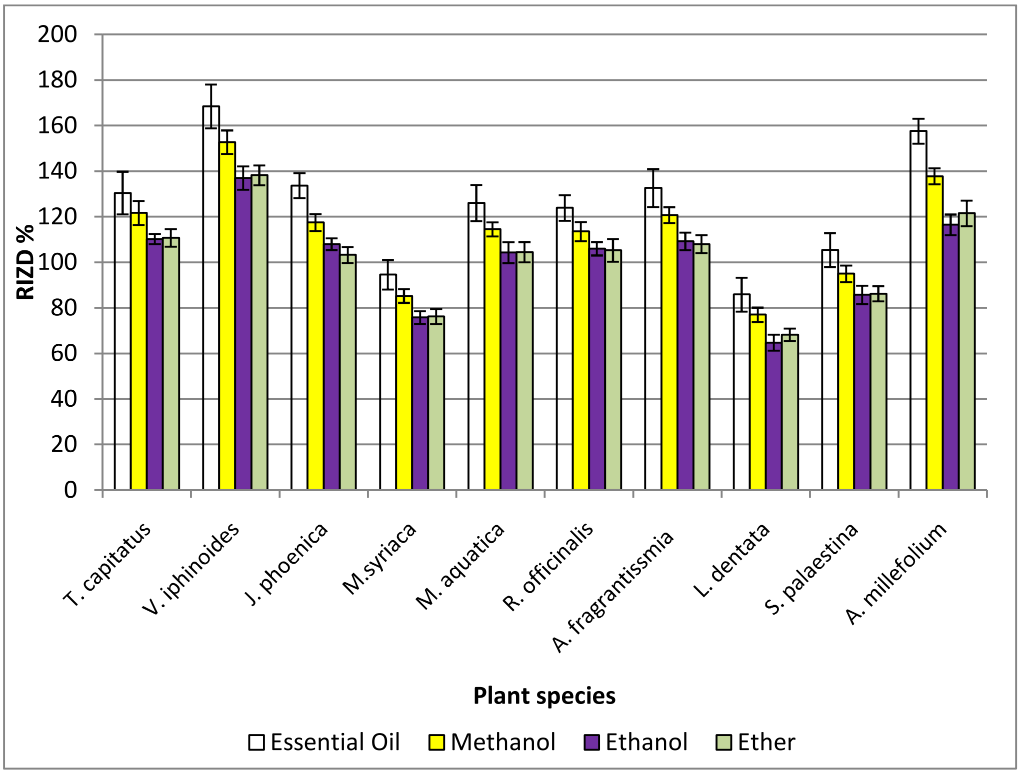


Figure 5: Antifungal activity of medicinal plant extracts and essential oils against *C. albicans*

Figure (6) shows the antifungal activities of plant extracts and oils against *A. niger*. The oils of *O. syriaca* and *M. aquatica* possessed 152.59 ± 4.34 and 152.59 ± 7.11% RIZD respectively compared to standard antifungal agent. The oil that showed the lowest activity is the *L. dentata* oil with RIZD = 67.24 ± 4.45 %. All the tested oils and plant extracts showed activity against *A. niger* with variable RIZD. The extracts of *M. syriaca* and *M. aquatica* possess the highest activity while the extracts of *L. dentata* showed the lowest RIZD.

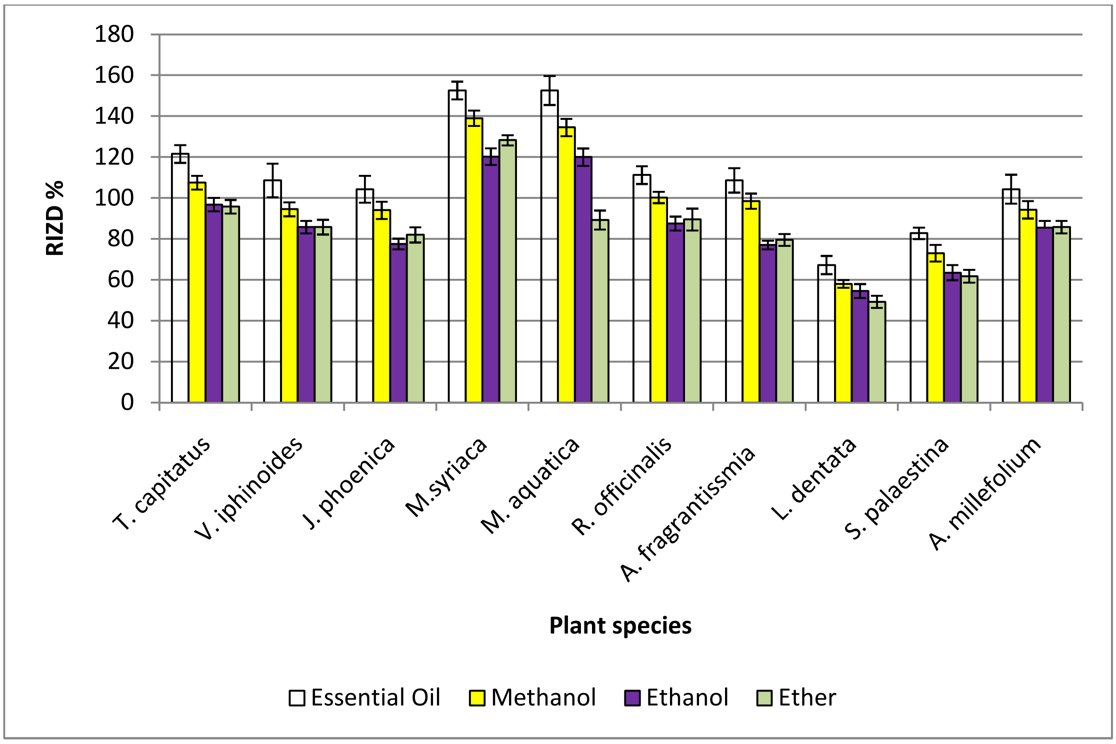


Figure 6: Antifungal activity of medicinal plant extracts and essential oils against *A. niger*.

The oils of *V. iphinoides* and *T. cpitatus* (figure 7) showed 105.77 ± 4.97and 102.88 ± 5.77 % RIZD compared to antifungal agent against *M. mehei*. While other essential oils showed RIZD lower than 100%. All the tested essential oils and plant extract showed activity against *M. mehei*. The oils were found to possesses higher activity against *M. mehei* than other plant extract.

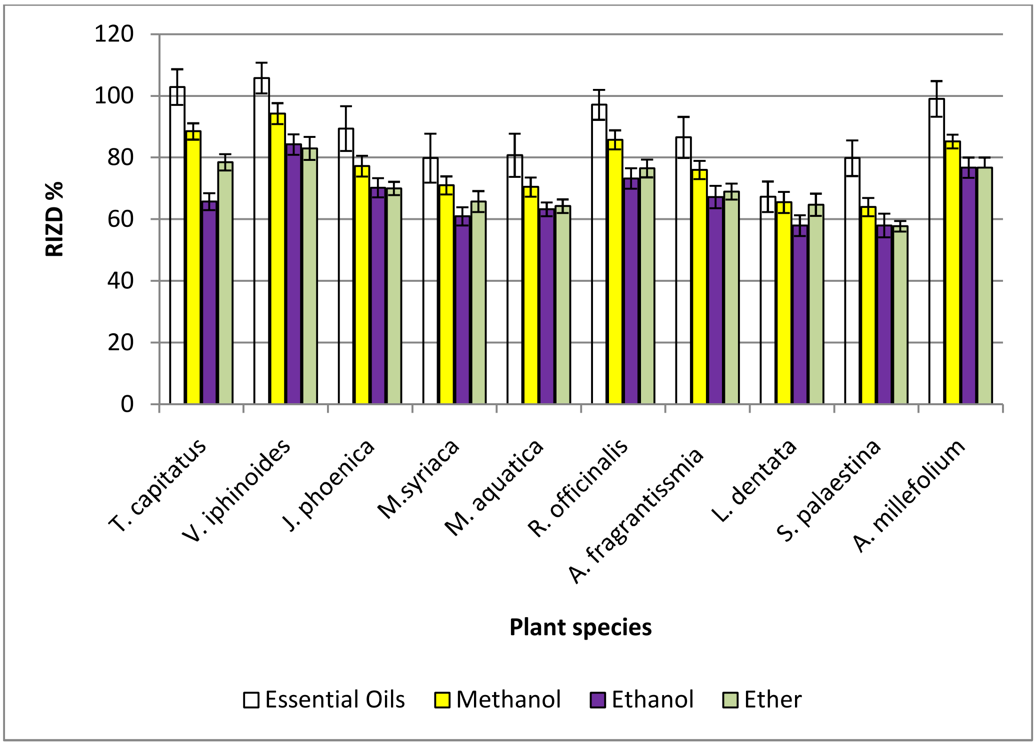


Figure 7: Antifungal activity of medicinal plant extracts and essential oils against *M. mehei*

Figure (8) shows the antifungal activities of plant extracts and oils against *T. mentagrophytes*. The essential oils of *T. cpitatus*, *V. iphinoides* and *L. dentata* gave 113.71 ± 4.84, 111.26 ± 6.18% and 108.06 ± 5.59 % RIZD values respectively compared to standard antifungal. All oils showed activity against *T. mentagrophytes* with RIZD ranging between 81.45 ± 4.84 % and 113.71 ± 4.84%. The results showed that the activities of the plant extracts against *T. mentagrophytes* were variable. The ether extract of *M. aquatica* showed RIZD = 66.00 ± 3.65 %, while the methanolic extract of *V. iphinoides* showed 103.25 ± 4.44 % RIZD.

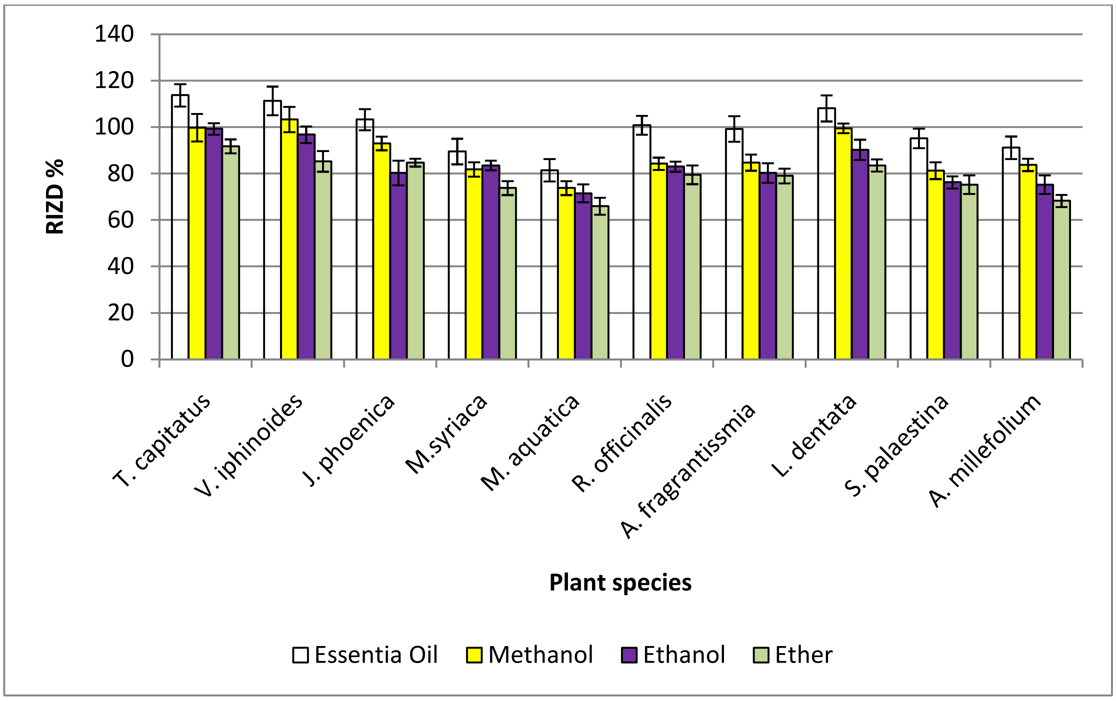


Figure 8: Antifungal activity of medicinal plant extracts and essential oils against *T. mentagrophytes*

The antifungal activity of the plant extracts and oils against *F. oxysporum* is shown in figure (9). All the tested oils and plant extracts exhibited activity against the fungus. The oil of *A. millefolium* showed significant activity (RIZD = 129.63 ± 4.66 %) compared to standard antifungal and other tested oils and extracts. The methanolic extract of *S. palaestiana* showed lower RIZD compared to oil for the same plant.

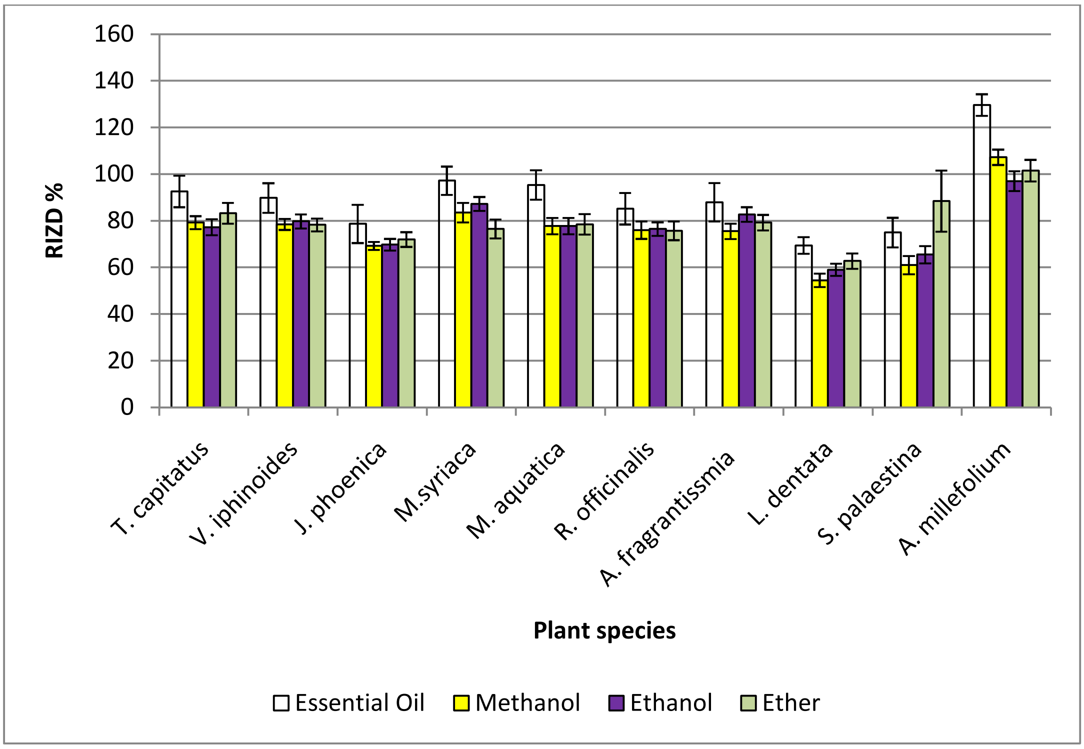


Figure 9: Antifungal activity of medicinal plant extracts and essential oils against *F. oxysporum*

Minimal Inhibitory Concentration

The MICs of the tested plant oils extracted by steam distillation are shown in table (1). As shown the MIC of oils varies depending on the plant species, for example the *M. aquatica* and *J. Phoenica* oils showed higher MICs comparing to standard antibacterial and antifungal agents. The oils of other tested plant species showed significantly lower MICs comparing to standards. The lowest MIC was observed for the *T. capitatus* oil that range between 2.5 - 5 µg/ml against tested bacteria, and 10 – 20 µg/ml against tested fungi. The MICs for standard antibiotic were ranged between 200 – 250 µg/ml, while they were ranged between 500- 750 µg/ml for antifungal.

Table (1): Minimal Inhibitory Concentrations (MICs) (µg/ml) of essential oils

	E. coli	***B. subtilis***	***S.aureus***	***P. aureginosa***	***C. albicans***	***A. niger***	***M. mehei***	***mentagrophytes***	***F. oxysprium***
***Achillea fragrentissmia* Forssk**	75	100	100	125	75	150	175	125	175
***Achillea millefolium* L.**	125	100	125	100	150	200	175	150	200
***Slavia palaestina* Benth.**	250	175	150	150	125	300	350	300	500
***Thymus capitatus* (L.) Hoffmanns & Link**	**5**	**2.5**	**5**	**5**	**10**	**15**	**15**	**10**	**20**
***Juniperus phoenica* L.**	500	600	500	450	800	800	750	1000	1500
***Mentha aquatic* L.**	750	500	750	500	500	1000	1000	750	1500
***Rosmarinus officinalis* L.**	50	75	50	50	100	125	150	125	150
Lavandulla dentata	75	75	50	75	100	150	200	150	200
Varthemia iphonoides	25	50	50	50	50	75	50	75	100
Majorana syriaca	**7.5**	**5**	**5**	**7.5**	**5**	**10**	**12.5**	**10**	**15**
Antibiotic	200	250	200	250	-	-	-	-	-
Antifungal	-	-	-	-	500	600	500	750	750

Effect of tested essential oils on fungal spore germination

The effect of essential oils concentrations on the fungal spore germination are shown in figures (10 – 19). All the tested essential oils significantly reduce the percent of spore germination; the reduction of spore germination was concentration dependent. The spore germination reduced as the concentration increase.

The effect of *T. capitatus* essential oil is shown in figure (10). It was observed that the *T. capitatus* essential oil reduced the fungal spore germination significantly. There was no spore germination of *T. mentagrophytes* at 15 µg/ml essential oil concentration. While no spore germination of *A. niger* and *M. mehei* was observed at concentration of 20 µg/ml oil, The *F. oxysporium* spore germination at this concentration was 9.75 ± 2.22 %.

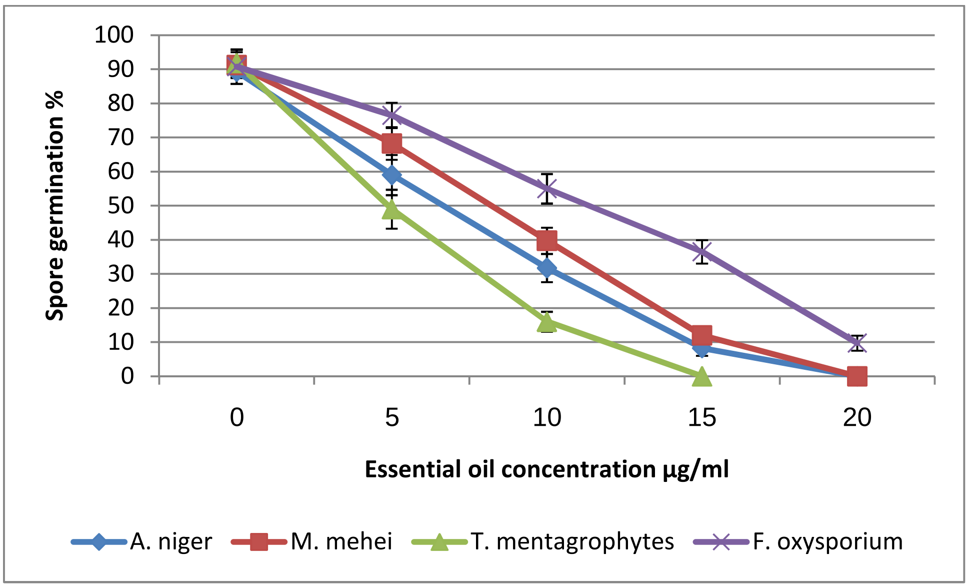


Figure 10: Effect of *T. capitatus* essential oil on spore germination of fungi

The spore germination of fungi was affected significantly by *A. millefolium* oil in a concentration dependent manner (Figure 11). The percent of germinated spore for *M. mehei* and *T. mentagrophytes* was 0 % at 200 µg/ml essential oil concentration. At this concentration there were 10.25 ± 4.03 % and 8.25 ± 2.2 % of germinated spores for *A. niger* and *F. oxysporium* respectively. A reduction in more than 50% spores germination for all fungi at 100µg/ml of oil concentration was observed.

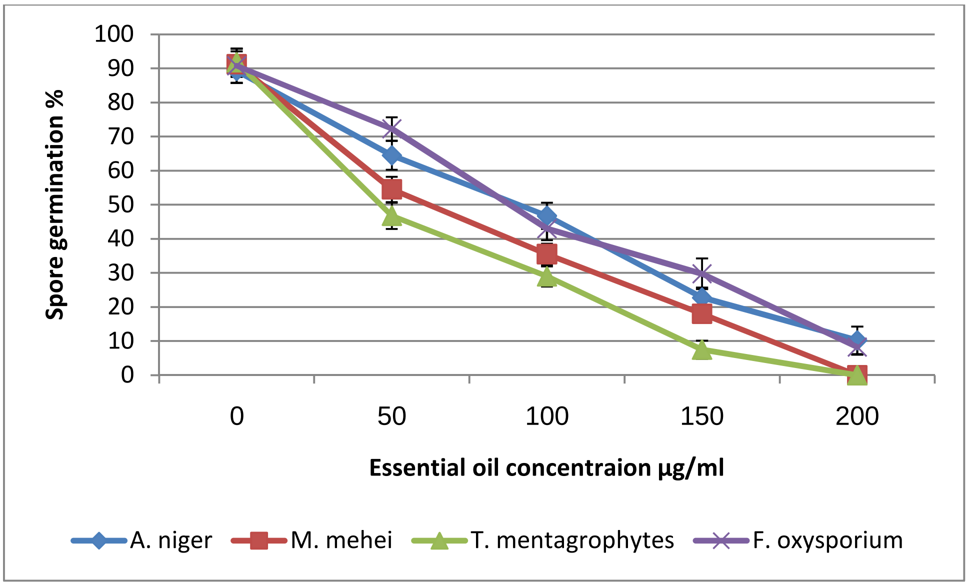


Figure 11: Effect of *A. millefolium* essential Oil on Spore germination of fungi

The effect of *V. iphinoides* essential oil on the fungal spore germination percent is shown in figure (12). As shown there was more than 50% reduction in the number of germinated spores for all fungi at 50 µg/ml essential oil concentration. At 75 µg/ml essential oil concentration no germination was observed for *M. mehei*. For *T. mentagrophytes* and *A. niger* the essential oil concentration required to prevent spore germination was 100 µg/ml. There was 9 ± 2.94% germinated spore for *F. oxysporium* at the previous concentration.

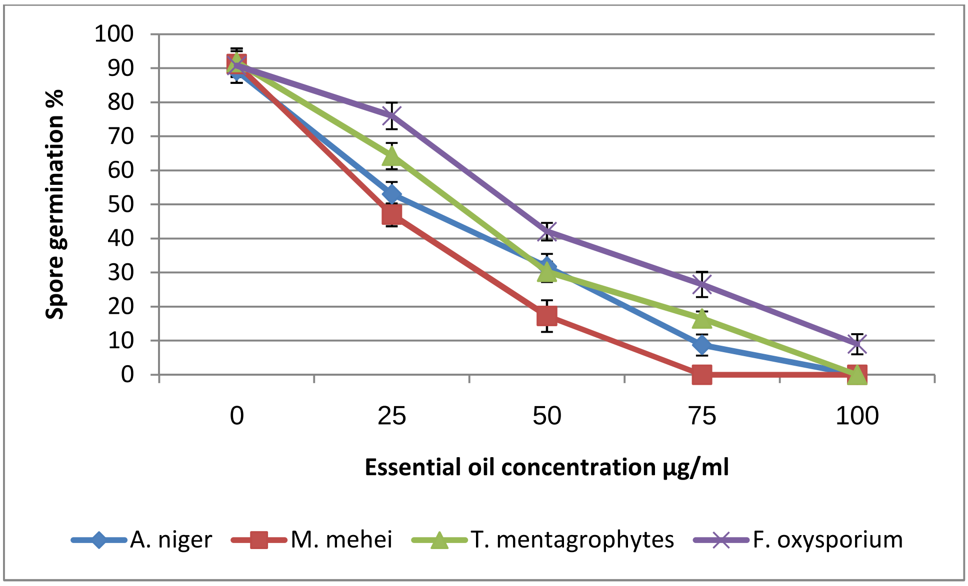


Figure 12: Effect of *V. iphinoides* essential oil on spore germination of fungi

The effect of *J. phoenica* essential oil on fungal spore germination percent is shown in figure (13). As shown there was more than 50% reduction in the number of germinated spores for all fungi at 750 µg/ml essential oil concentration. At 1000 µg/ml essential oil concentration no germination was observed for *M. mehei* and *A. niger*. For *T. mentagrophytes* higher essential oil concentration was required to prevent spore germination, this concentration was 1250 µg/ml. There was 7.75 ± 2.75% germinated spore for *F. oxysporium* at the 1500 µg/ml essential oil concentration.

Figure (14) shows the effect of *O. syriaca* oil on fungal spore germination. As shown, there was no spore germination for *A. niger*, *M. mehei* and *T. mentagophytes* at 15 µg/ml essential oil concentration occurred, while there was spore germination for *F. oxysporium* at this concentration = 8 ± 2.16 %.

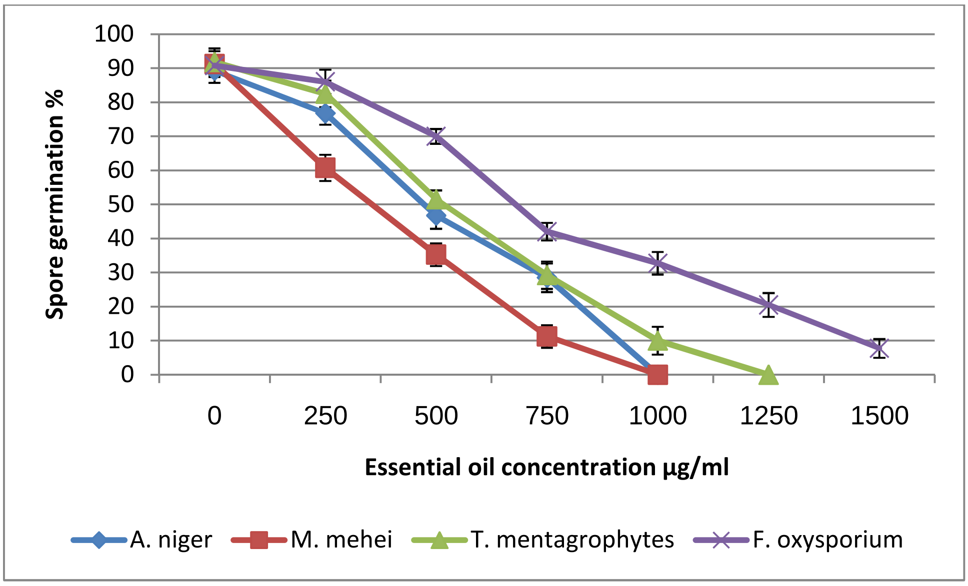


Figure 13: Effect of *J. phoenica* essential Oil on Spore germination of fungi

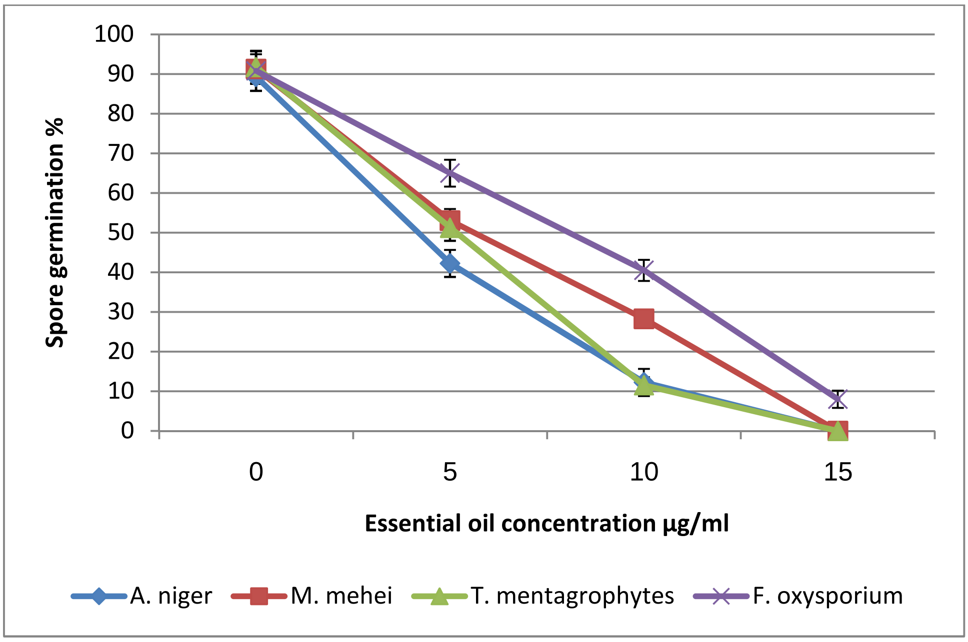


Figure14: Effect of *O. syriaca* essential oil on spore germination of fungi

The effect of *M. aquatica* essential oil on fungal spore germination percent is shown in figure (15). The percentage of spore germination reduced significantly as the concentration of oil increased. At 1000 µg/ml essential oil concentration no germination was observed for *T. mentagrophytes*. For *A. niger* and *M. mehei* higher essential oil concentration was required to prevent spore germination, this concentration was 1500 µg/ml, There was 9 ± 2 % geminated spore for *F. oxysporium* at this essential oil concentration.

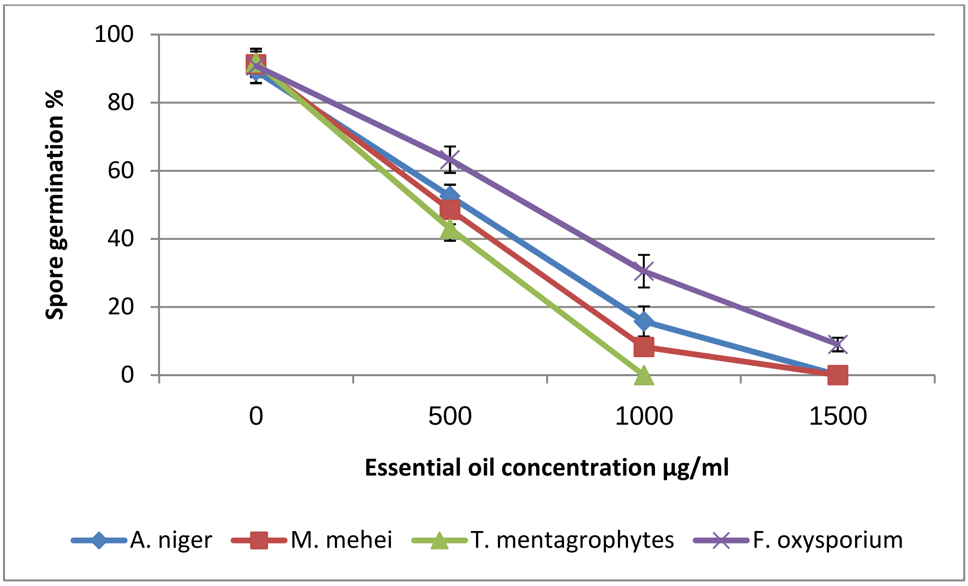


Figure 15: Effect of *M. aquatica* essential Oil on Spore germination of fungi

Figure (16) shows the Effect of *R. officinalis* essential oil on fungal spore germination. As shown there was more than 50% reduction in the number of germinated spores for all fungi at 100 µg/ml essential oil concentration. The results were shown that there was no observed spore germination for *A. niger* and *T. mentagophytes* at 150 µg/ml essential oil concentration, while there was 16 ± 4.08 and 7.5 ± 3.11 % germinated spore for *M. mehei* and *F. oxysporium* respectively at this concentration.

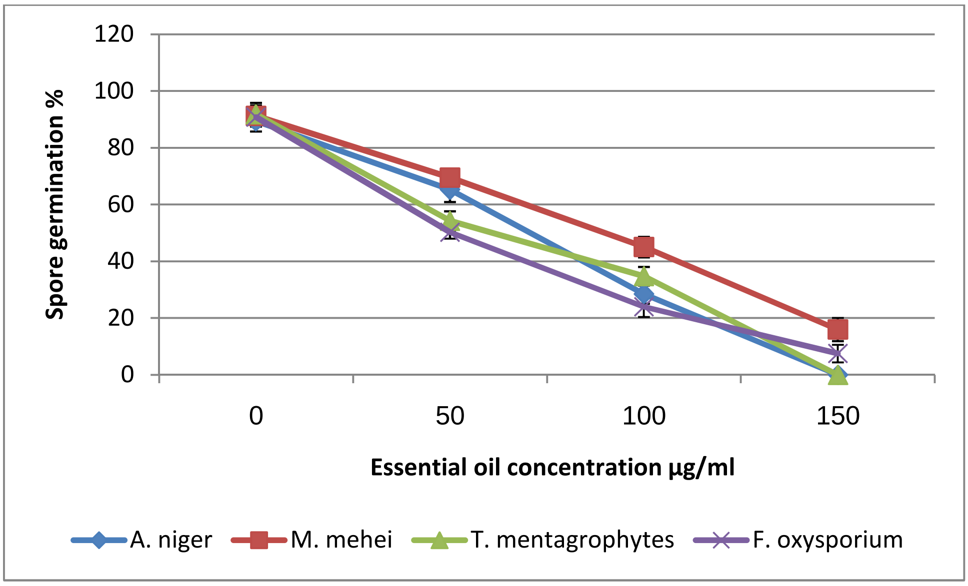


Figure16: Effect of *R. officinalis* essential Oil on Spore germination of fungi

The percent of spore germination was reduced significantly with increase of *A. fragrantissmia* oil concentration (Figure 17). The percent of germinated spore for *T. mentagrophytes* was 0 % at 150 µg/ml essential oil concentration. At this concentration there were 8.5 ± 2.89, 20.25 ± 2.75 % and 27.25 ± 3.59 % of germinated spores for *A. niger* and *F. oxysporium* and *M. mehei* respectively.

Figure (18) show the effect of *L. dentata* essential oil on the percent of fungal spore germination. As result shown there was less than 50% germinated spore for *A. niger* , *F. oxysporium* and *T. mentagrophytes* at 100 µg/ml oil concentration, while this concentration yield 52 ± 2.75 % of spore germination for *M. mehei*. There was no observed spore germination for the both fungi *A. niger* and *T. mentagrophytes* at 200 µg/ml essential oil concentration. There was 6.75 ± 3.3 and 13.25 ± 3.86 % geminated spore for the other two fungi at the previous oil concentration.

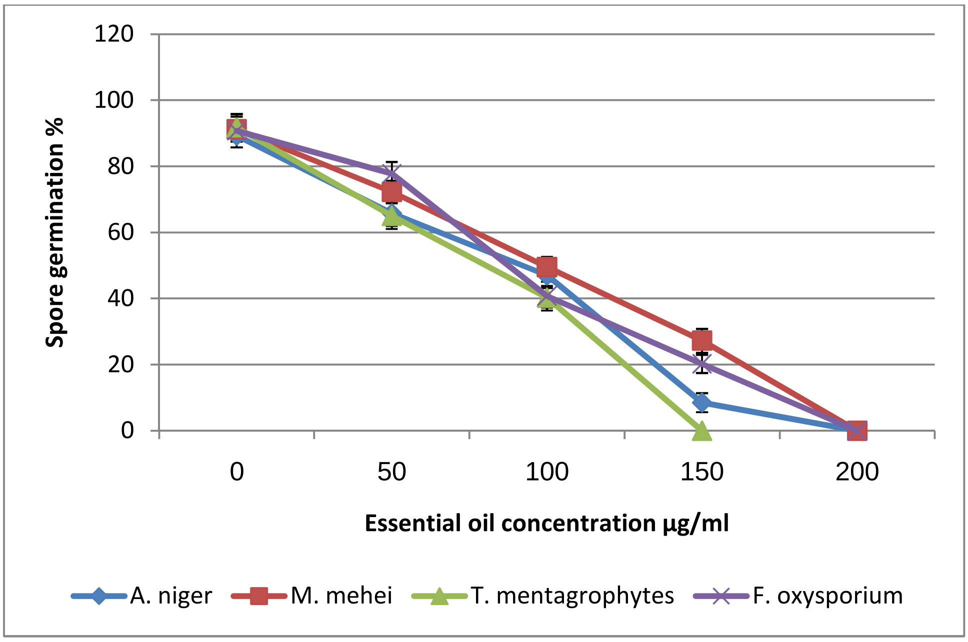


Figure 17: Effect of *A. fragrantissmia* essential Oil on Spore germination of fungi

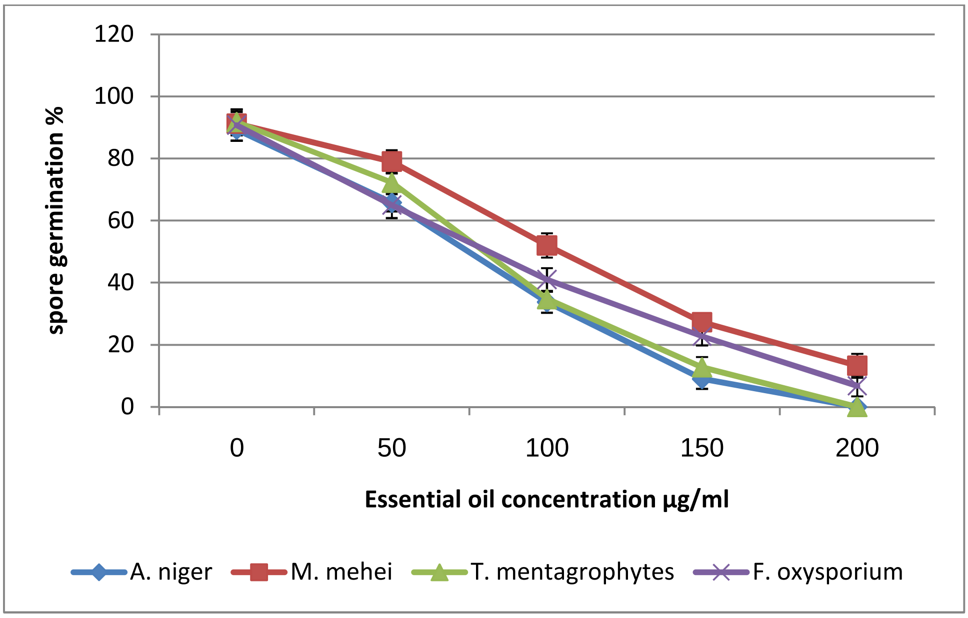


Figure 18: Effect of *L. dentata* essential Oil on Spore germination of fungi

Figure (19) shows the effect of *S. palestina* essential oil on the percent of fungal spore germination. A less than 50% germinated spore for *A. niger, T. mentagrophytes* and *M. mehei* at 200 µg/ml essential oil concentration was noticed. However, at this concentration there was 7.75 ± 2.99 % germinated spore for *F. oxysporium* was shown. There was no spore germination occurred for *A. niger*, *T. mentagrophytes* and *M. mehei* at 400 µg/ml essential oil concentration. There was 42.25 ± 3.4 and 7.75 ± 2.75 % geminated spore for *F. oxysporium* at 400 and 500 µg/ml essential oil concentration, respectively.

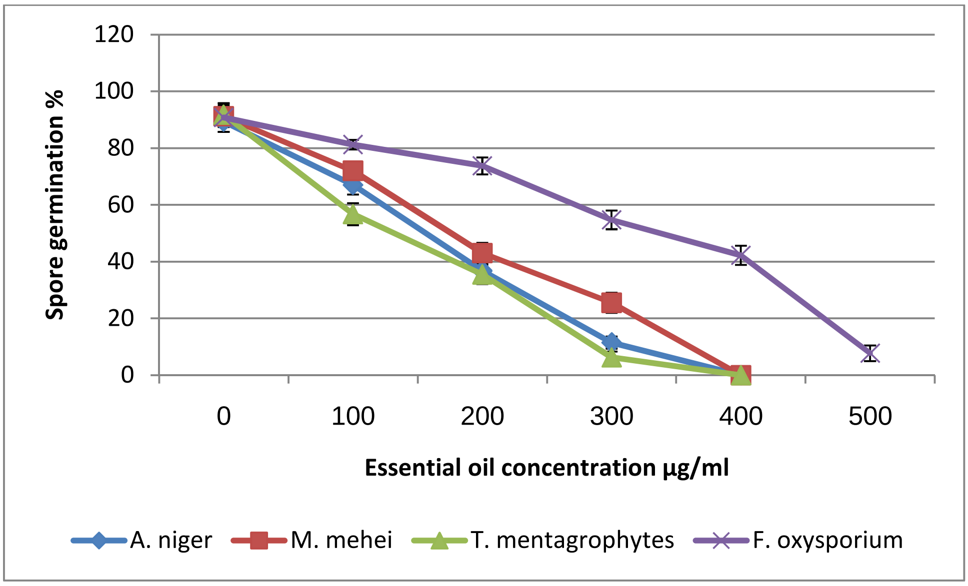


Figure19: Effect of *S. palestina* essential Oil on Spore germination of fungi

Effect of fatty acid on the antifungal activity of essential oils

Figure (20) show the effect of fatty acid addition on the antifungal activity of essential oils. As shown all the fungus species grow at normal range with the presence of essential oils (MICs concentrations) with addition of fatty acid. The dry weight is comparable to the control dry weight (Fungus without essential oils addition). No growth was observed at the presence of essential oils without fatty acid addition.

No effect was observed for the addition of casamino acid and nucleotide mixture. The result showed that there was no growth at the presence of essential oils with the addition of casamino acid or nucleotide mixture.

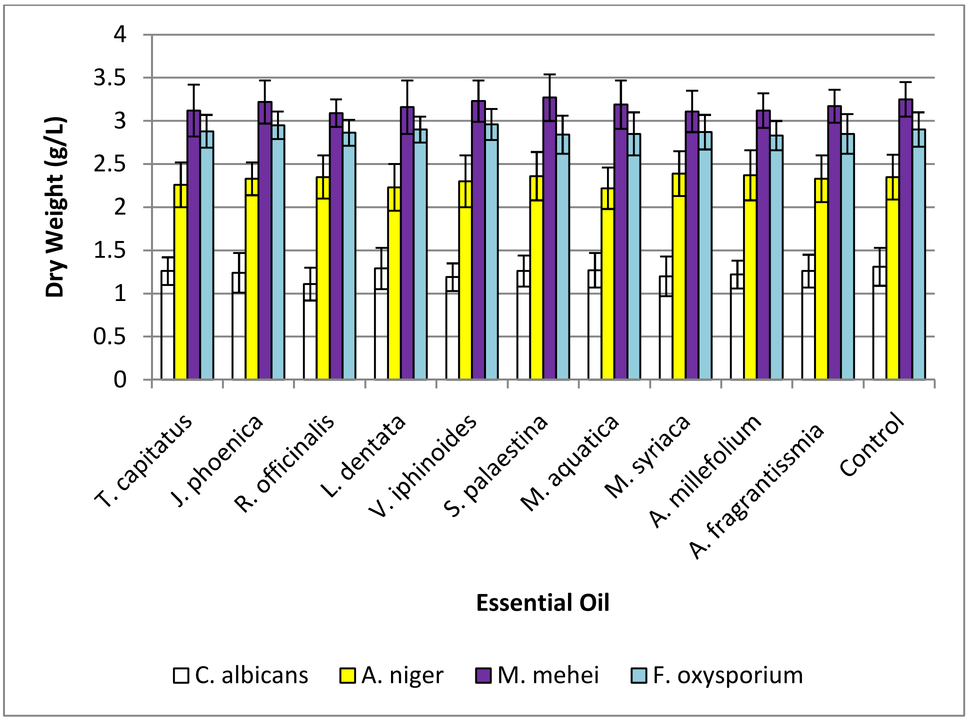


Figure 20: Effect of the fatty acid addition on the antifungal activity of essential oils.

Antioxidant activity

2,2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging activity

The antioxidant activity of oils of *T. capitatus, J. phoenica*, *R. officinalis* and *L. dentata* was assessed using DPPH radical scavenging assay. DPPH radical scavenging activity was observed with all the tested oils (figure 21), the essential oils showed higher activity compared to vitamin C (Figure 22), there was more than 50% DPPH activity inhibition at 10 µg/ml oils concentrations of *T. capitatus, J. phoenica* and *L. dentata* comparing to 25 ± 1.63 % activity inhibition by vitamin C at this concentration. The results showed that DPPH activity inhibition at 20 µg/ml oils concentration exceeds 80%

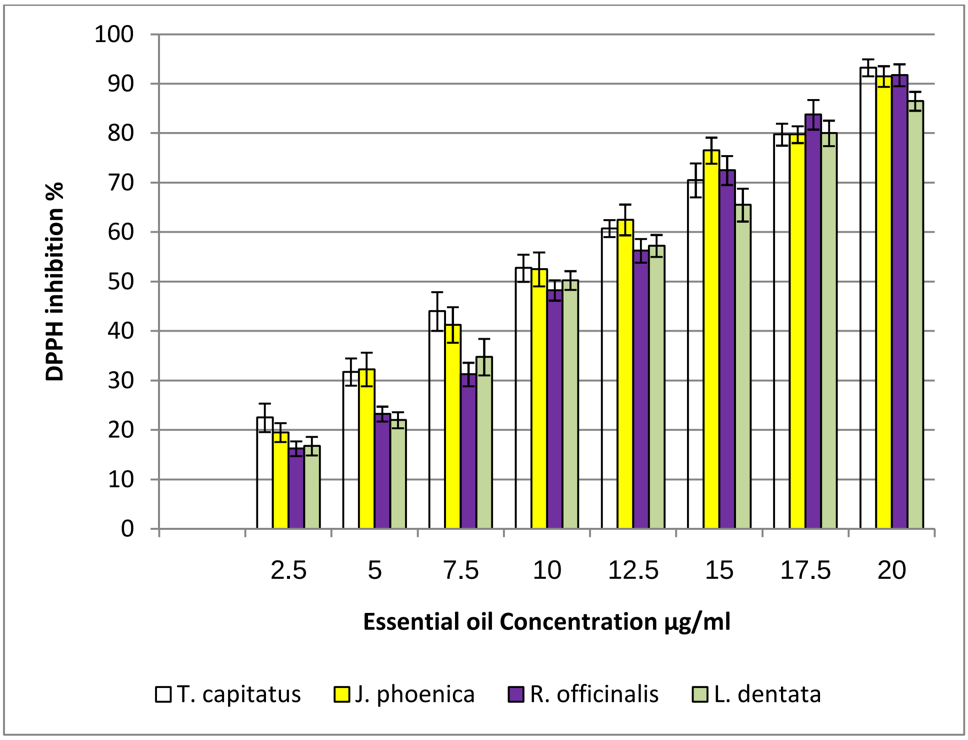


Figure 21: Inhibition of DPPH by the essential oils of *T. capitatus, J. Phoenica, R. officinalis* and *L. dentata*.

Figure (22) show the DPPH activity inhibition by the essential oils of *S. palaestina, V. iphinoide, M. aquatica* and Vitamin C. As shown DPPH radical scavenging activity was observed in all essential oils, the essential oils of *S. palaestina* and *V. iphinoide* showed significantly lower activity comparing to vitamin C at 20 µg/ml, the inhibition of DPPH was 36.25 ± 3.42 and 44 ± 2.75% inhibition at this essential oils concentration respectively comparing to 54.75 ±1.89 inhibition by vitamin C. There was more than 50% DPPH activity inhibition at 25 µg/ml concentrations of the three essential oils and vitamin C. The results were shown that DPPH activity inhibition at 40 µg/ml essential oils concentration exceed 85%

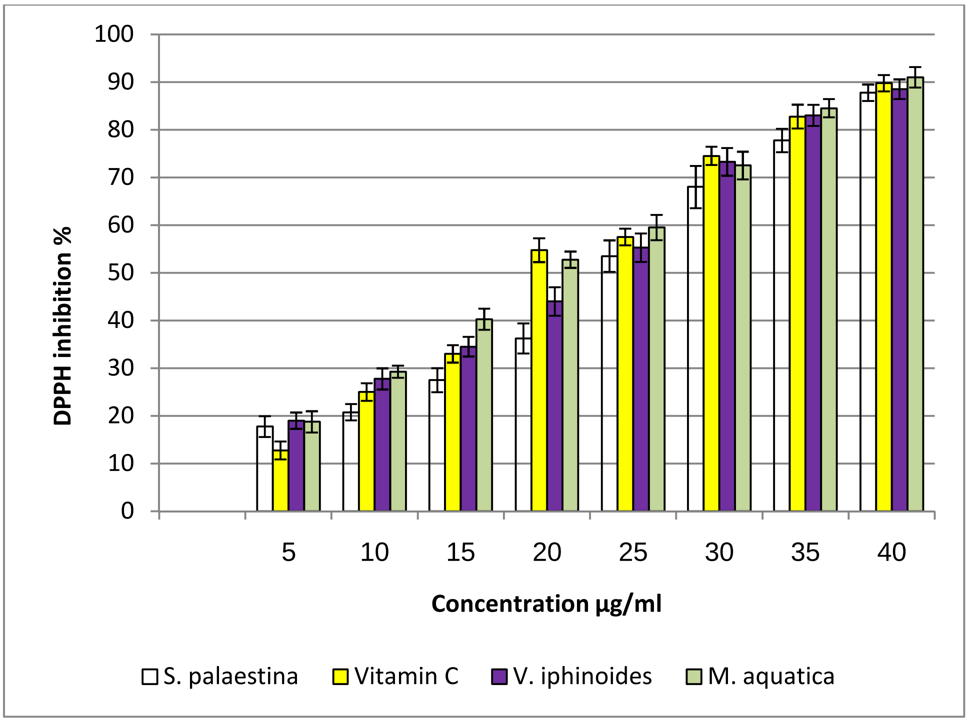


Figure 22: Inhibition of DPPH by the essential oils of *S. palaestina*, *V. iphinoide, M. aquatica* and Vitamin C.

The Inhibition of DPPH by the essential oils of *M. syriaca, A. fragrantissima* and *A. millefolium* is shown at figure (23). As shown all the essential oils possess DPPH radical scavenging activity at concentration ranging from 20 – 160 µg/ml. Comparing to vitamin C figure (22) there was significantly low activity for the three essential oils at 20 µg/ml essential oils concentration. There was more than 50% DPPH scavenging activity inhibition by the three essential oils at 100 µg/ml concentration. At 160 µg/ml essential concentration the DPPH inhibition exceed 80%.

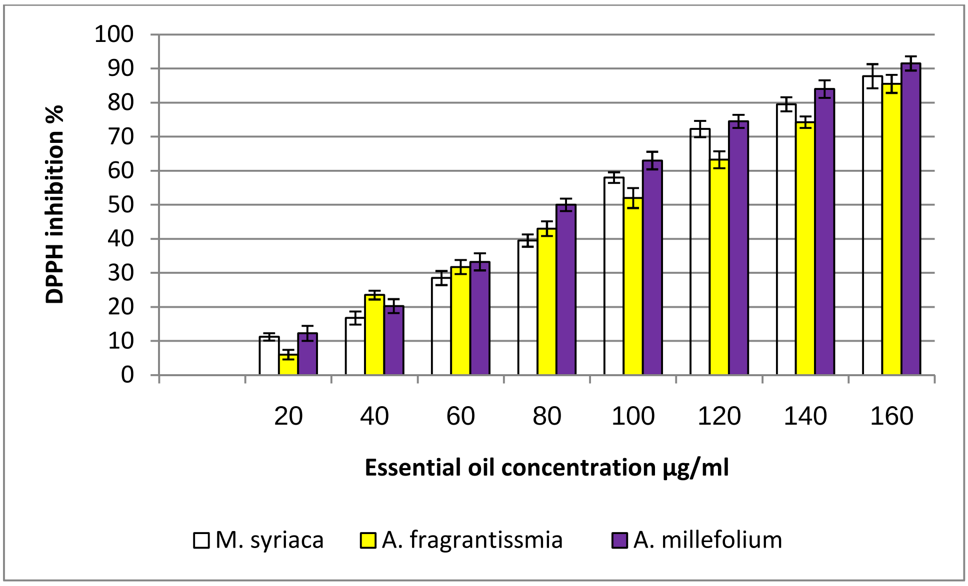


Figure 23: Inhibition of DPPH by the essential oils of *M. syriaca*, *A. fragrantissima* and *A. millefolium*

As shown above (figure 20 – 22) all essential oils possess DPPH scavenging activity inhibition, the inhibition increase with the essential oils concentration increase. The plant essential oils vary in their ability to inhibit DPPH scavenging activity.

Nitrogen Oxide (NO) Scavenging Activity

The nitrogen oxide scavenging activity of *T. capitatus*, *J. phoenica*, *L. dentata* and *R. officinalis* essential oils are shown in figure (24). The oils possessed NO scavenging activity at concentration ranging between 5 and 30 µg/ml essential oils concentration. The essential oil of *L. dentata* possess significant NO scavenging activity comparing to the standard antioxidant rutin (figure25) at 20 µg/ml concentration. The result were shown that there was 43.25 ± 1.71 % inhibition of NO scavenging activity by *L. dentata* essential at the previous concentration comparing to 33 ± 1.83 % inhibition by Rutin. The inhibition of NO scavenging activity by all essential oils was observed to be concentration dependent.

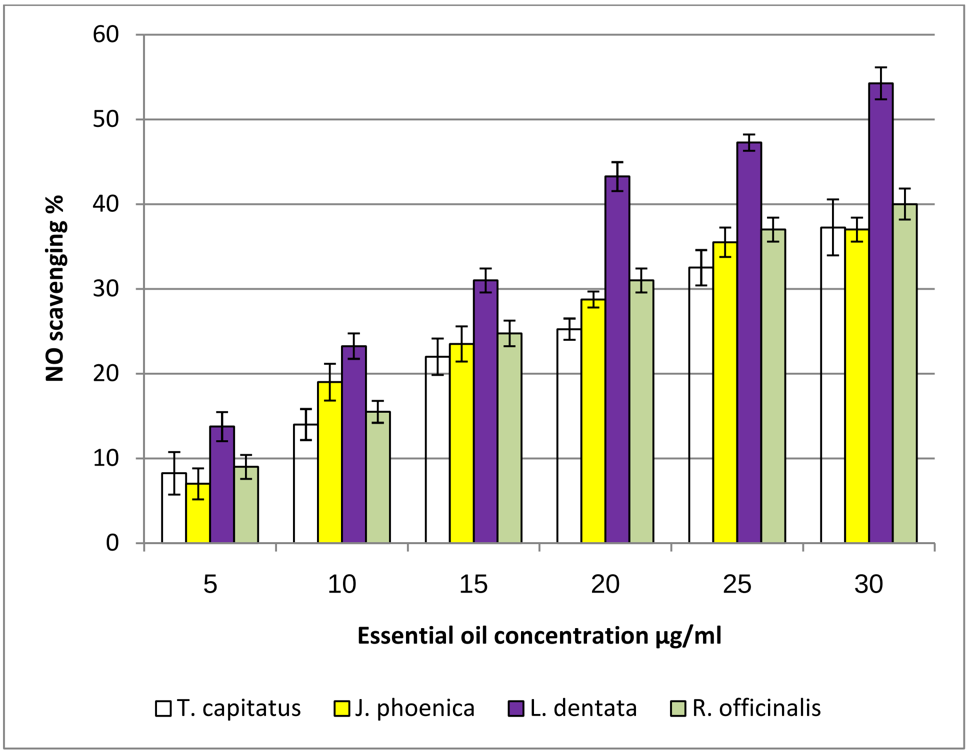


Figure 24: Nitrogen Oxide (NO) scavenging activity of *T. capitatus*, *J. phoenica*, *L. dentata* and *R. officinalis* essential oils.

The nitrogen oxide scavenging activity of *V. iphinoides*, *S. palaestina* and *M. aquatica* essential oils and Rutin are shown in figure (25). All the essential oils possess NO scavenging activity at concentration ranging between 10 and 60 µg/ml essential oils concentration. The essential oil of *V. iphinoides* possesses comparable NO scavenging activity with the standard antioxidant rutin at 20, 30 and 40 µg/ml concentration. The result were shown that there was more than 50 % inhibition of NO scavenging activity by *V. iphinoides* and *S. palaestina* essential oil at 40 and 50 µg/ml concentration, respectively. The inhibition of NO scavenging activity by all essential oils was observed to be concentration dependent. The results were shown that *M. aquatica* essential oil have significantly lower NO scavenging inhibition at 40 -60 µg/ml concentration comparing to other essential oils and standard antioxidant rutin.

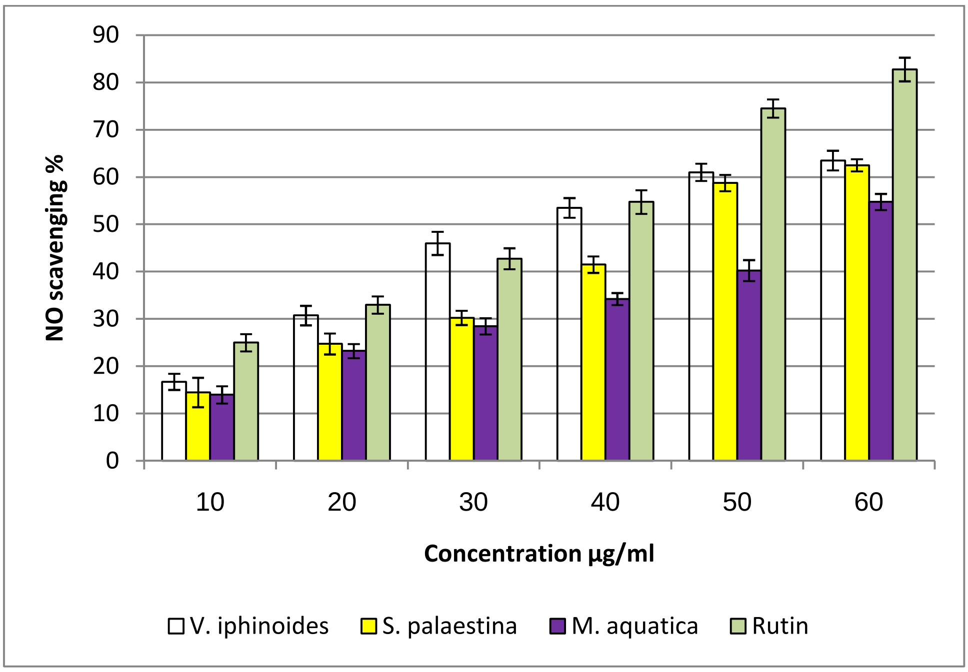


Figure 25: Nitrogen Oxide (NO) scavenging activity of *V. iphinoides*, *S. palaestina* and *M. aquatica* essential oils and Rutin respectively.

Figure (26) show NO scavenging activity by *M. syriaca, A. millefolium* and *A. fragrantissma* essential oils. All the essential oils possess NO scavenging activity at concentration ranging between 25 and 150 µg/ml essential oils concentration. The essential oils possess significantly lower activity NO scavenging activity inhibition comparing with the standard antioxidant rutin at 50 µg/ml concentration. The result were shown that there was more than 50 % inhibition of NO scavenging inhibition by *A. millefolium* and *A. fragrantissma* essential oil at 100 µg/ml concentration. The results were shown that *M. syrica* essential oil have significantly higher NO scavenging inhibition at 150 µg/ml concentration comparing to other essential oils. At 25 µg/ml *M. syrica* essential oil possess significantly lower inhibition comparing to other essential oils.

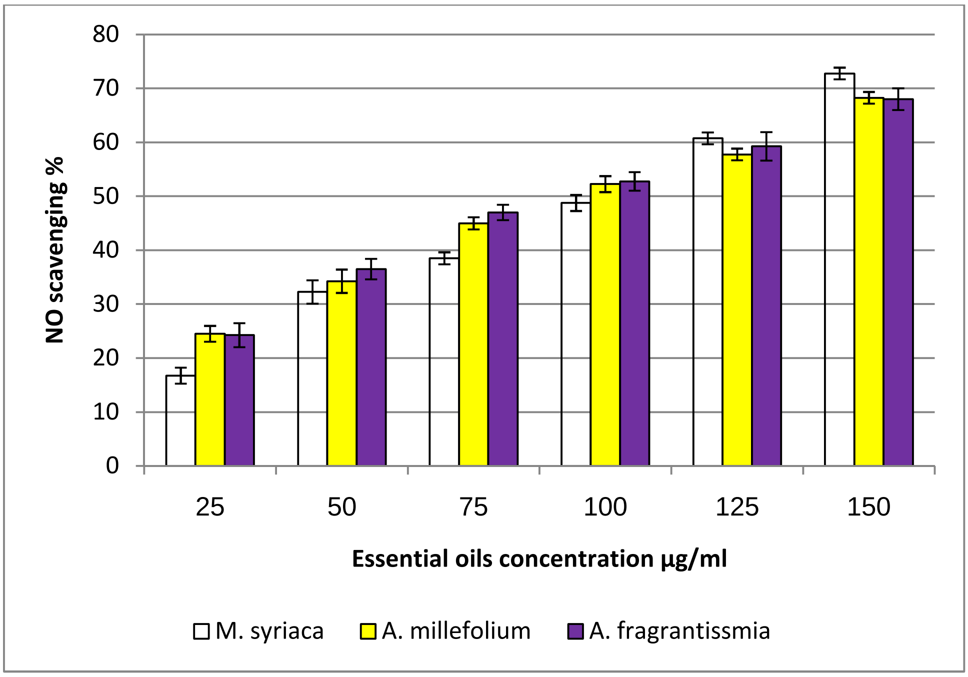


Figure 26: Nitrogen Oxide (NO) scavenging activity of *M. syriaca, A. millefolium and A. fragrantissima* essential oils respectively.

Superoxide anion scavenging activity

The superoxide anion scavenging activity of *T. capitatus, J.phoenica, R. officinalis* and *L. dentata* essential oils are shown in figure (27). As shown all these essential oils possess activity at 5 µg/ml oils concentrations, at this concentration no significant differences in activity were observed by *T. capitatus, J. phoenica,* and *L. dentata* essential oils. The oils were showed significantly higher activity at 20 µg/ml concentrations compared to curcumin shown in figure 28. The oil of *L. dentata* possesses significantly higher activity at 10 – 30 µg/ml concentration compared to other oils and curcumin. While the results were shown that the essential oil of *R. officinalis* gave significantly the lowest activity comparing to other essential oils.

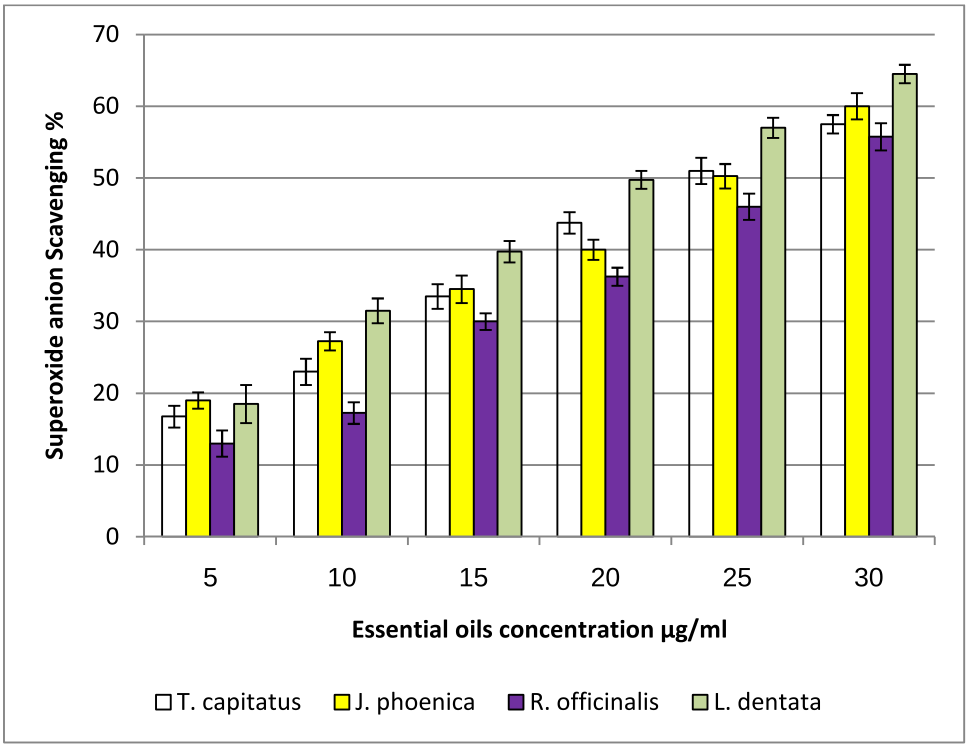


Figure 27: Superoxide anion scavenging activity of *T. capitatus, J. phoenica, R. officinalis* and *L. dentata* essential oils.

Figure (28) shows the superoxide anion scavenging activity of *V. iphinoides, S. palaestina,* and *M. aquatica* essential oils and Curcumin. The results were shown that all the essential oils had superoxide anion scavenging activity at concentration dependent manner. It was observed that *V. iphinoides* essential oil possesses significantly higher activity at 20 and 30 µg/ml comparing to curcumin, it gave 40 ± 1.83 and 49.5 ± 2.65 % scavenging at these concentration respectively comparing to 33 ± 1.83 and 32.75 ± 2.22 % for curcumin at the same concentrations. There were no significant observed between the essential oils and curcumin at 10 µg/ml concentrations. At 50 µg/ml concentrations there was more than 50% superoxide anion scavenging for all oils and curcumin, the essential oil of M. aquatica possess significantly lower activity comparing to other essential oils and curcumin at this concentration. While, the curcumin possess significantely higher activity at 60 µg/ml concentration.

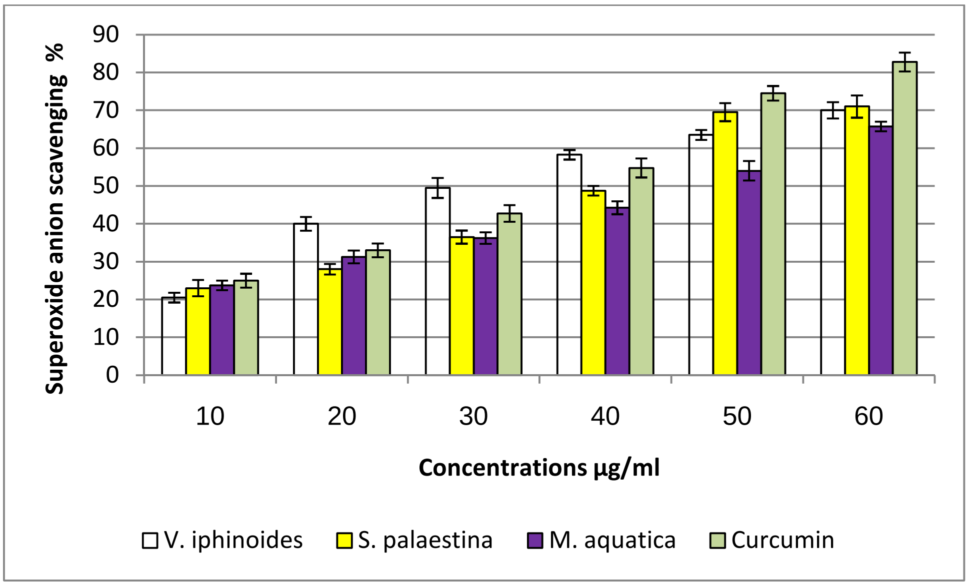


Figure 28: Superoxide anion scavenging activity of *V. iphinoides, S. palaestina, M. aquatica* essential oils and Curcumin.

The superoxide anion scavenging activity of *M. syriaca*, *A. millefolium* and *A. fragrantissima* essential oils and are shown in figure (29). The results were shown that all the essential oils had superoxide anion scavenging activity at concentration dependent manner. It was observed that *M.syriaca* essential oil possesses significantly the lowest activity at all comparing to other essential oils and curcumin. The essential oil of *A. millefolium* possesses significantly higher activity comparing to other essential oils at 125 and 150 µg/ml concentration , the result were shown that the scavenging activity at these concentrations was 73.25 ± 2.06 and 74 ± 2.16 % respectively, comparing to 64.5 ± 1.29 and 67 ± 1.29 for *A. fragrantissima* essential oil at the same concentration respectively. There were no significant differences observed between the essential oils of *A. fragrantissima* and *A. millefolium* at 25 - 100 µg/ml concentrations. At 75 µg/ml concentrations there was more than 50% superoxide anion scavenging by *A. fragrantissima* and *A. millefolium* essential oils. The essential oil of *M.syriaca* possess more than 50% superoxide scavenging at higher concentration (150 µg/ml).

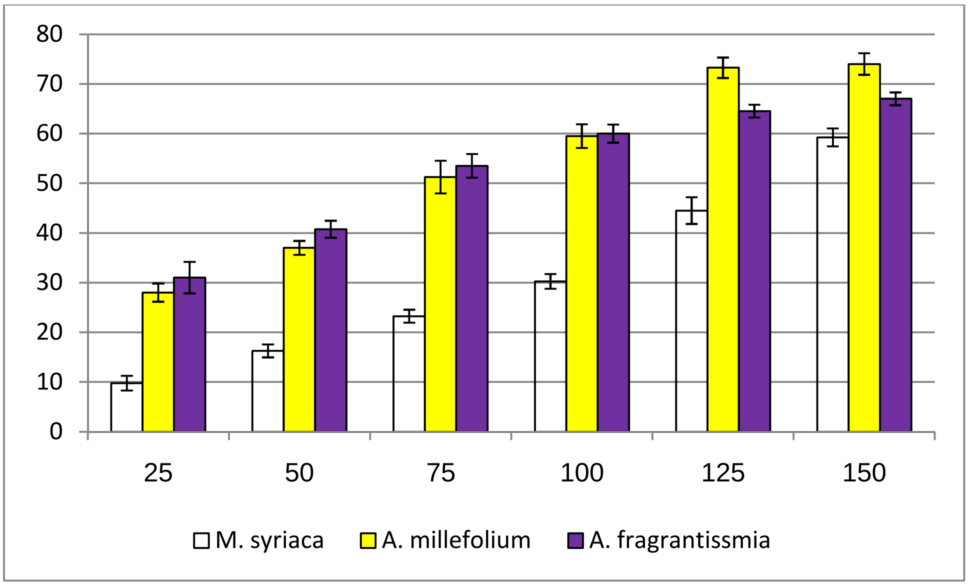


Figure 29: Superoxide anion scavenging activity of *M. syriaca*, *A. millefolium* and *A. fragrantissima* essential oils.

Reducing power

The reducing power of *T. capitatus, J.phoenica, R. officinalis* and *L. dentata* essential oils were shown in figure (30). As shown the reducing power increased in essential oils concentration dependent manner. The four essential oils showed significantly higher reducing power comparing to Butylated hydroxy toluene (BHT) figure (31). At 10 µg/ml essential oil concentration the observed absorbance was 0.46 ± 0.02, 0.45 ± 0.02, 0.54 ± 0.02 and 37 ± 0.02 for *T. capitatus, J.phoenica, R. officinalis* and *L. dentata* essential oils respectively comparing to 0.26 ± 0.02 for BHT at the same concentration. The result showed that *L dentata* essential oil possessed significantly lower reducing power at 5 – 15 µg/ml concentration comparing to other essential oils. The essential oil of *R. officinalis* had significantly higher reducing power comparing to other oils at 25 µg/ml concentration. No significant reducing power differences were observed at 20 µg/ml concentration.

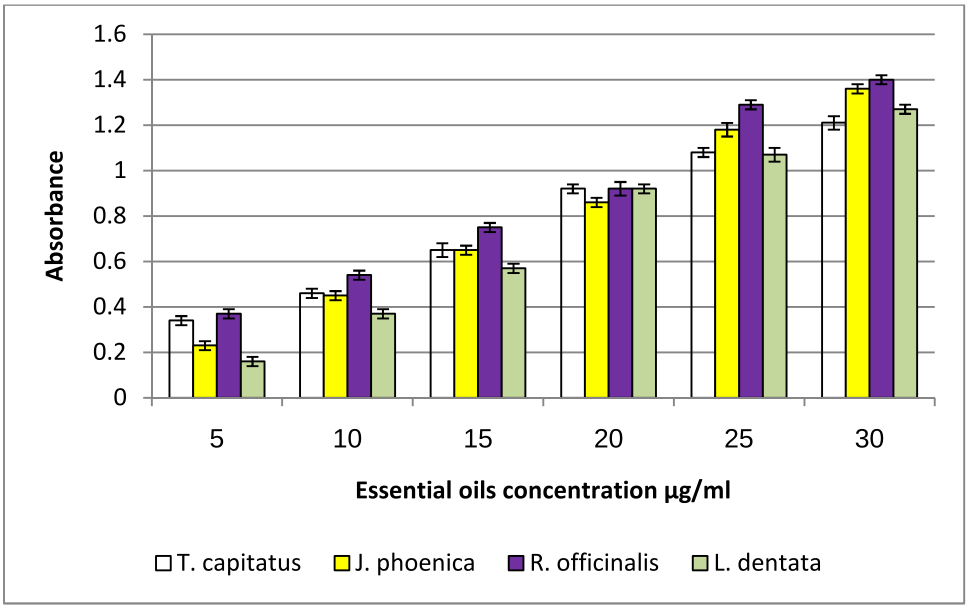


Figure 30: Reducing power assay of *T. capitatus, J. phoenica, R. officinalis* and *L. dentata* essential oils.

Figure (31) show the reducing power assay result of *V. iphinoides*, *S. palaestina* and *M. aquatica* essential oils and BHT. As shown *M. aquatica* essential oil possessed significantly higher reducing power at 10 µg/ml concentration comparing to BHT, the absorbance was 0.26 ± 0.02 and 0.16 ± 0.02 for essential oil and BHT respectively. At 30 µg/ml concentration *V. iphinoides* showed significantly higher reducing power comparing to other essential oils and BHT, the absorbance was 0.72 ± 0.03 for *V. iphinoides* comaoring to 0.54 ± 0.02 , 0.52 ± 0.02 and 0.6 ± 0.02 for *S. palaestina* , *M. aquatica* essential oils and BHT respectevily. While the results were shown that at 50 and 60 µg/ml, there was significantly higher reducing power possessed by *M. aquatica* essential oil comparing to other essential oils and BHT, the observed absorbance at these concentration was 1.18 ± 0.02 and 1.36 ± 0.02 respectively comparing to 0.94 ± 0.02, 0.85 ± 0.03 and 0.9 ± 0.02 for *S. palaestina*, *M. aquatica* essential oils and BHT at 50 µg/ml respectively, and 1.1 ± 0.03, 1.177 ± 0.02 and 1.22 ± 0.02 at 60 µg/ml concentrations. All the essential oils possessed increase reducing power in concentration dependent manner.

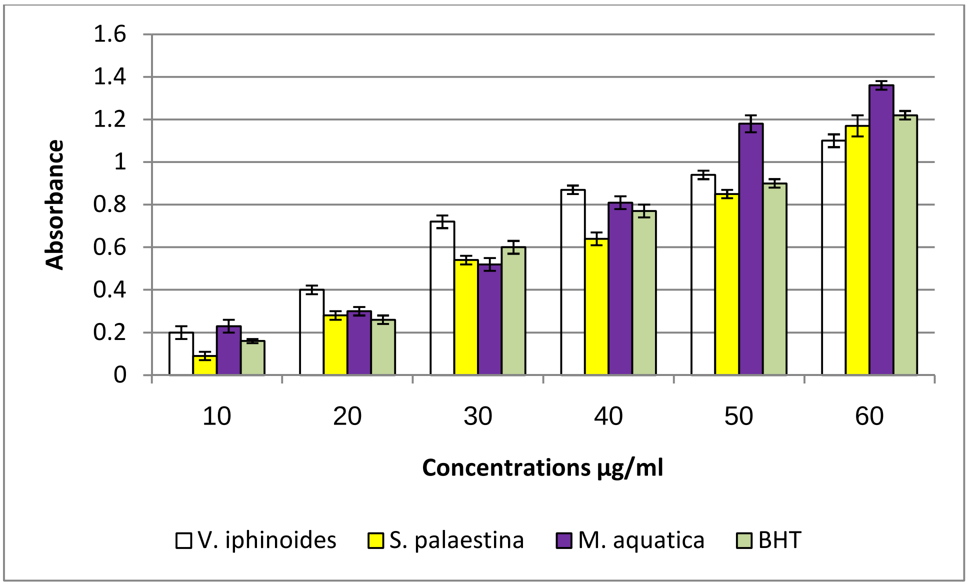


Figure 31: Reducing power assay of *V. iphinoides, S. palaestina* and *M. aquatica* essential oils.

Reducing power assay of *M. syriaca*, *A. millefoulim* and *A. fragrantissima* essential oils are shown in figure (32). The results were shown that all essential oils possessed increasing reducing power at concentrations ranging between 25 - 150 µg/ml, this increase was observed to be concentration dependent. The essential oil of *M. syriaca* showed significantly higher reducing power than the other two oils at 25, 50 and 75 µg/ml concentrations, the absorbance at these concentrations was 0.26 ± 0.02, 0.45 ± 0.03 and 0.6 ±0.02 respectively, comparing to 0.17 ± 0.01, 0.23 ± 0.02 and 0.39 ± 0.03 for *A. millefoulim,* and 0.08 ± 0.01, 0.14 ± 0.02 and 0.35 ± 0.03 for *A. fragrantissima.* At 150 µg/ml concentration the essential oil of *A. millefoulim* showed higer activity comparing to the other two oils, while *A. fragrantissima* essential oil showed significantly lower reducing power comparing to other oils. All the three essential oils showed lower reducing power comparing to BHT (figure 30).

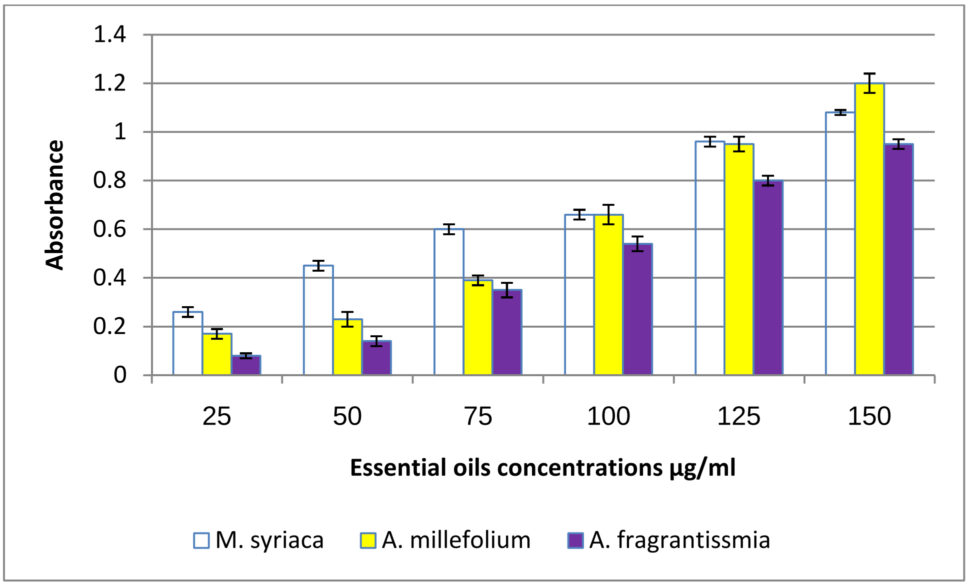


Figure 32: Reducing power assay of *M. syriaca, A. millefoulim* and *A. fragrantissima* essential oils.

Lipid peroxidation:

Figure (33) shows the results of lipid preoxidation assay of essential oils measured by Inhibition concentration 50 (IC50) (µg/ml). As shown the essential oils of *R. officinals*, *J. phoenica* and *L dentata* possessed stronger activity comparing to other essential oils and Vitamin E. The IC50 for these essential oils was observed to be 25 ± 1.87, 18.75± 2.17 and 23± 1.87 µg/ml respectively, comparing to 63.25 ± 2.95 µg/ml for vitamin E. The essential oils of *A. millefolium* and *A. fragrantissima* showed significantly lower activity (higher IC50) comparing to Vitamin E and other essential oils.

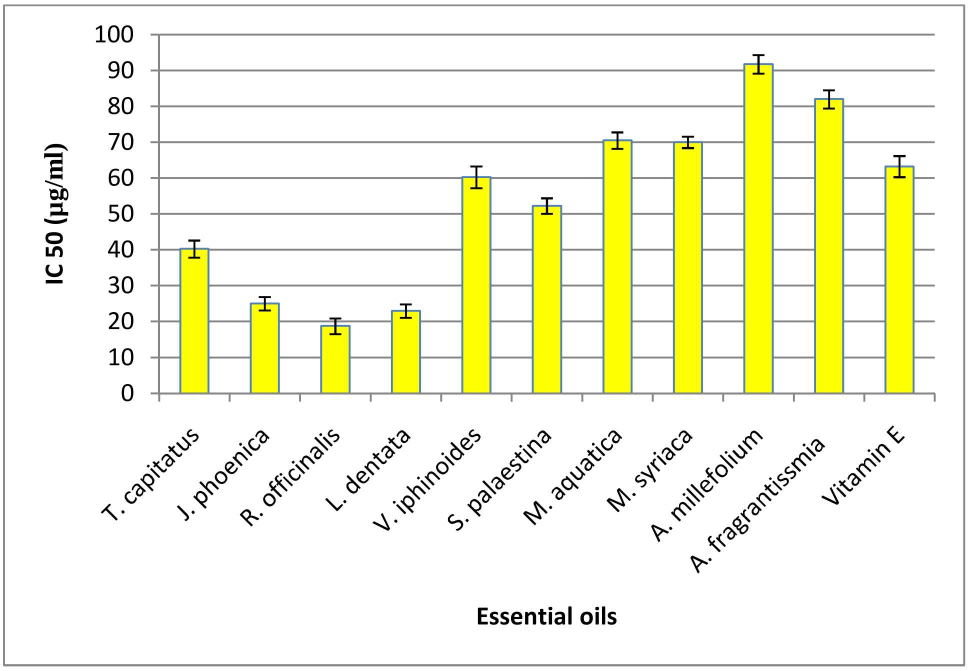
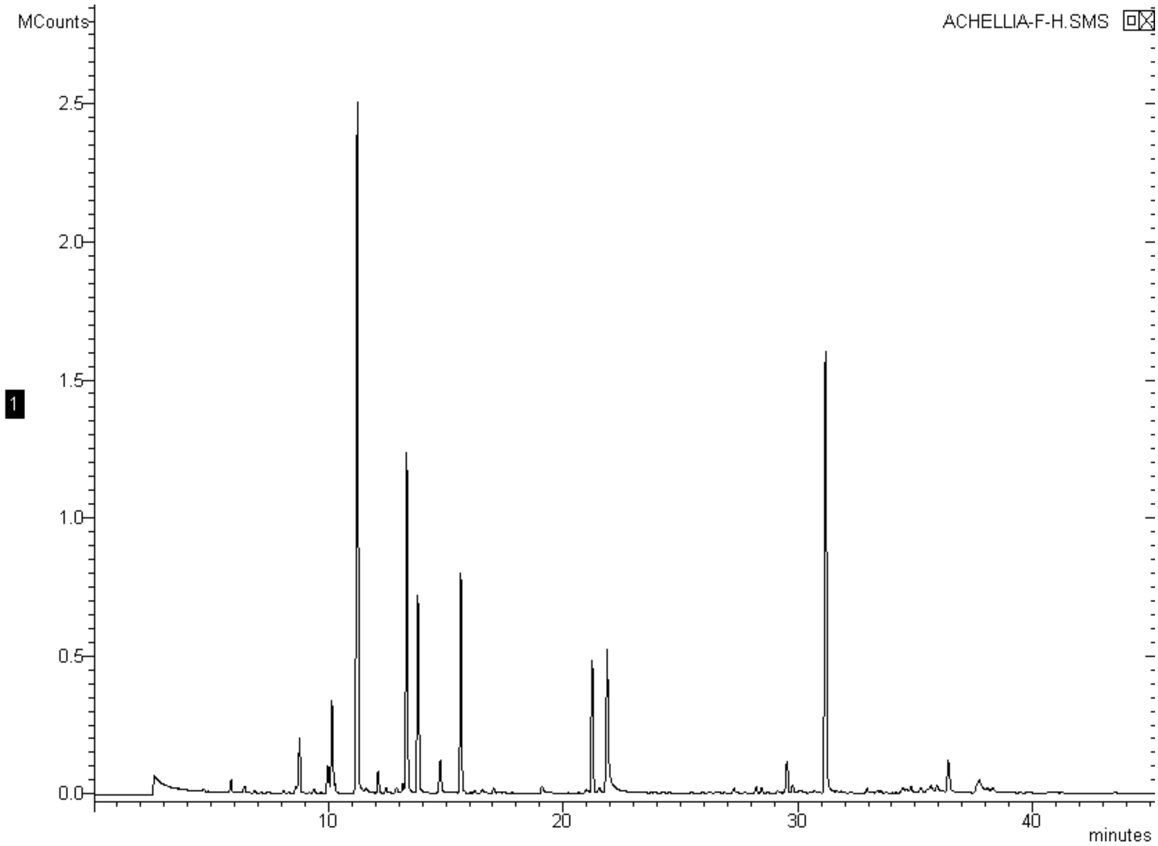


Figure 33: The lipid preoxidation of essential oils measured by Inhibition concentration 50 (µg/ml)

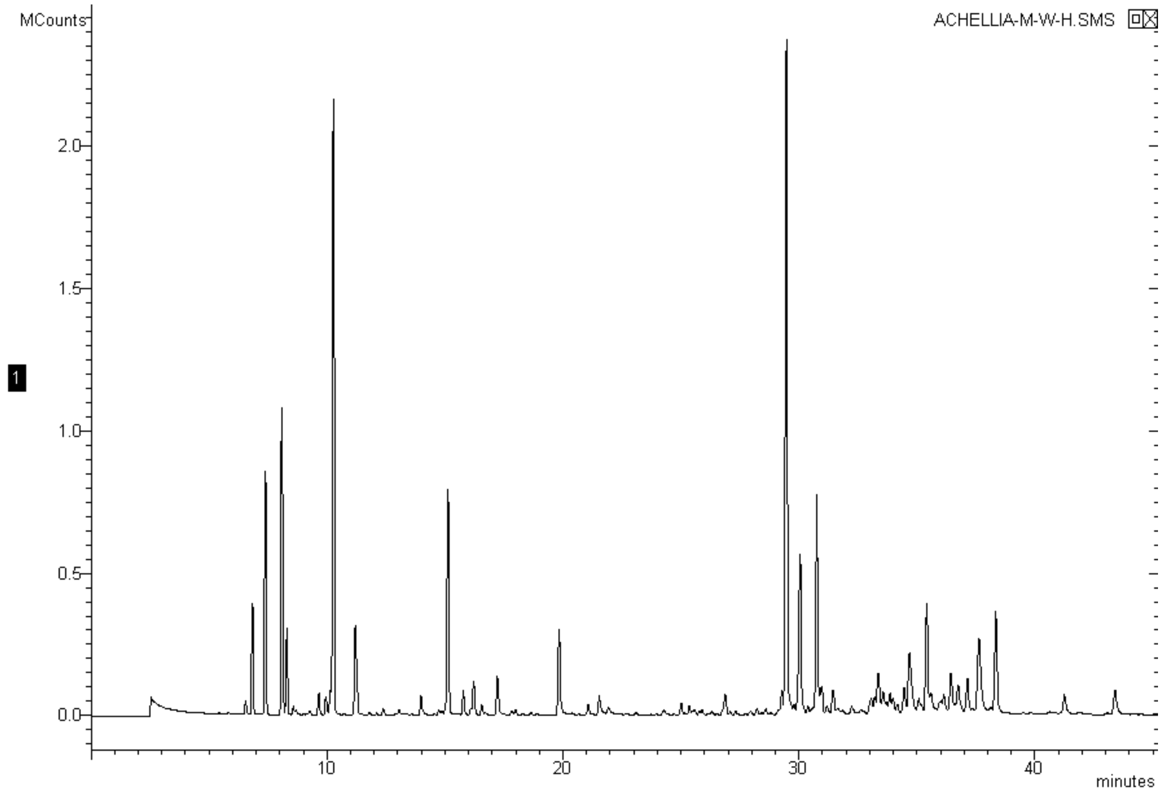
Chemical Composition of Essential Oils

The GC-MS chromatograms of the essential oils

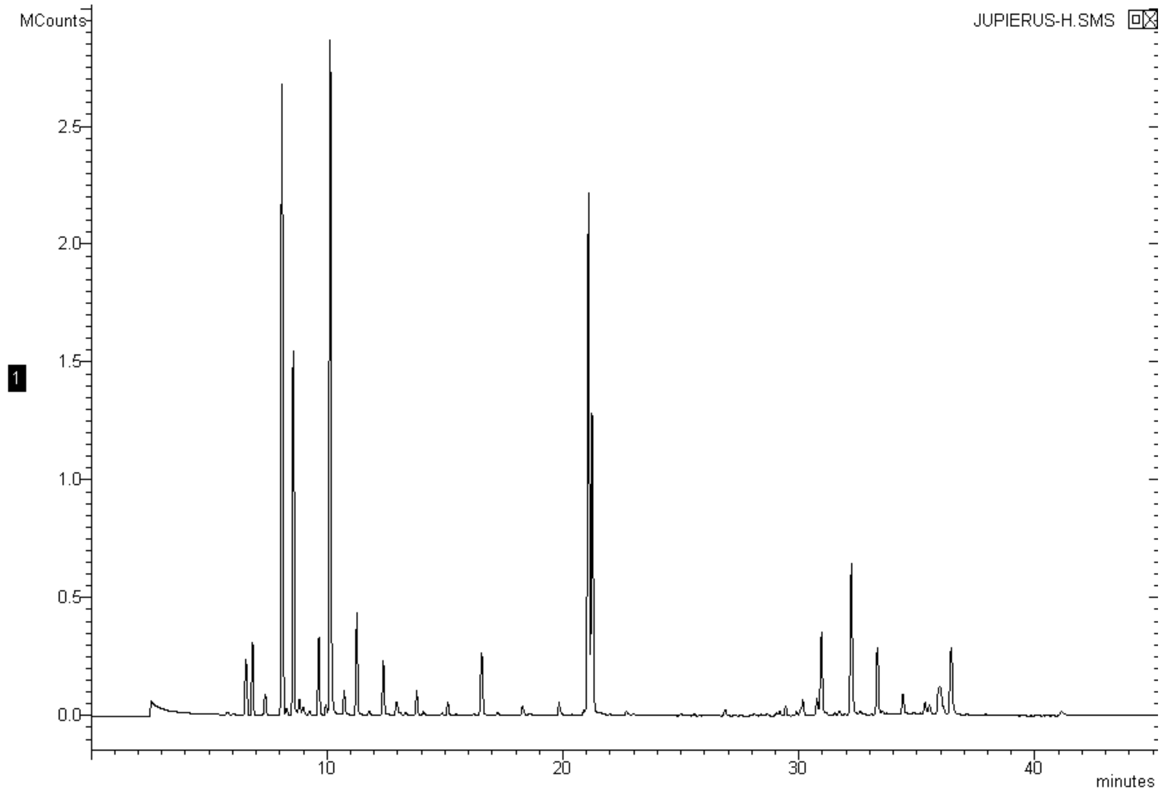
The chromatograms of the tesyed oils are shown in figure 34.



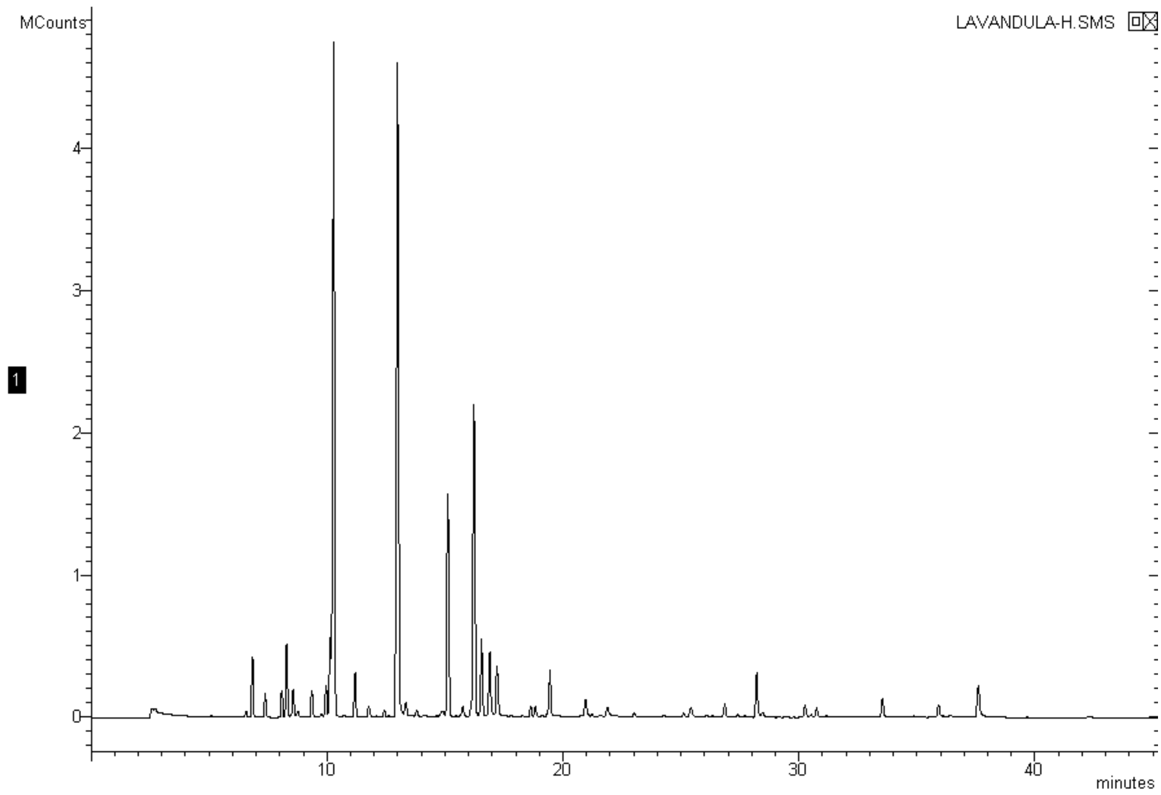
a- *Achillea fragrantissima*



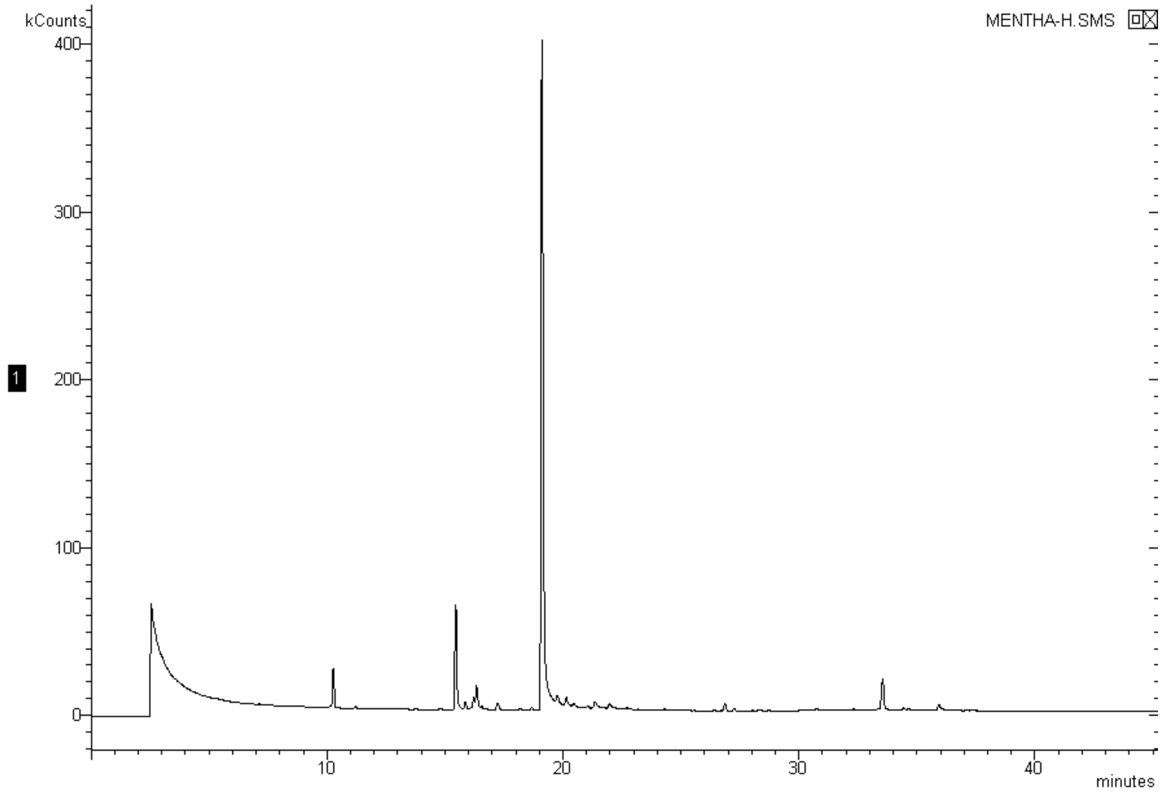
b- *Achillea millifolium*



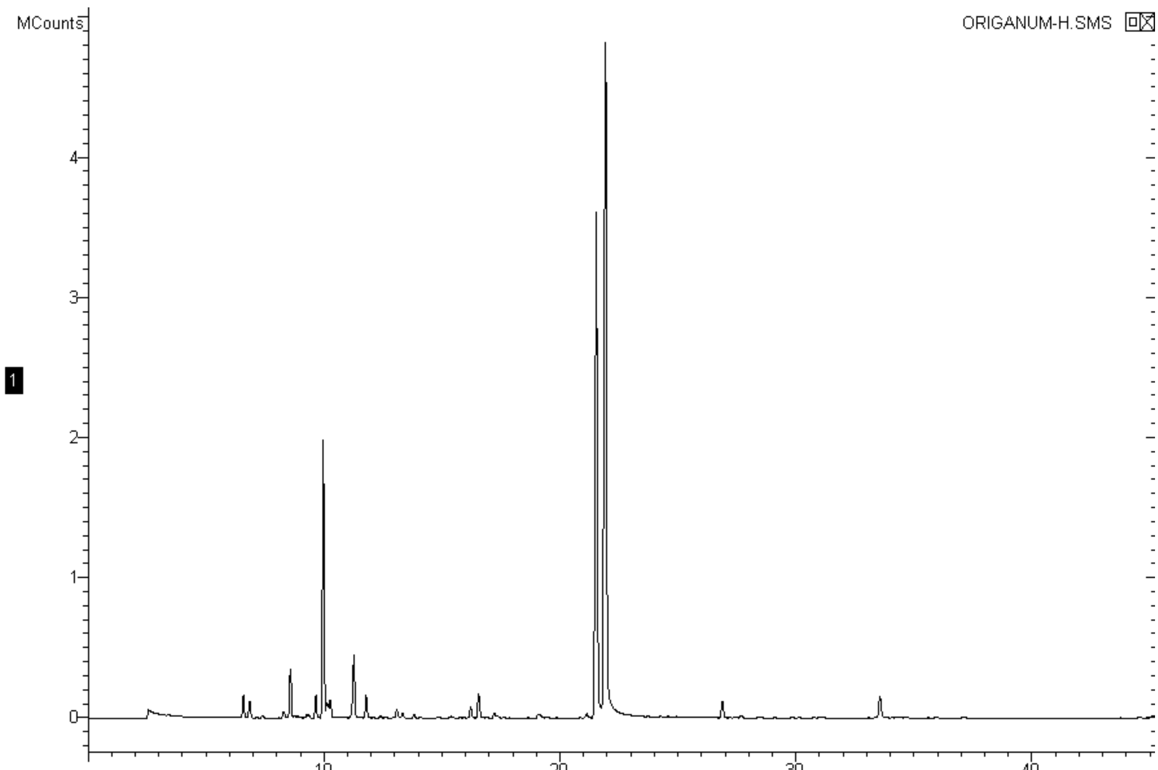
c- *Juniperus phoenica*



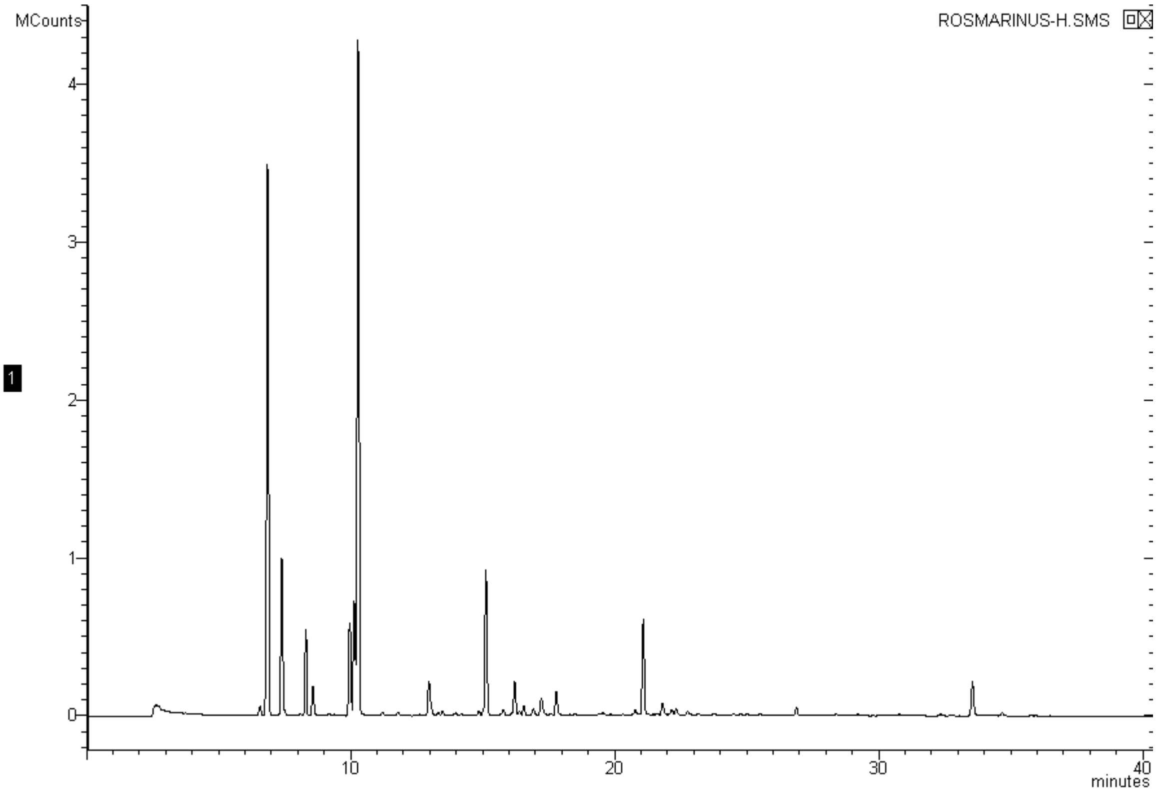
d- *Lavandula dentata*



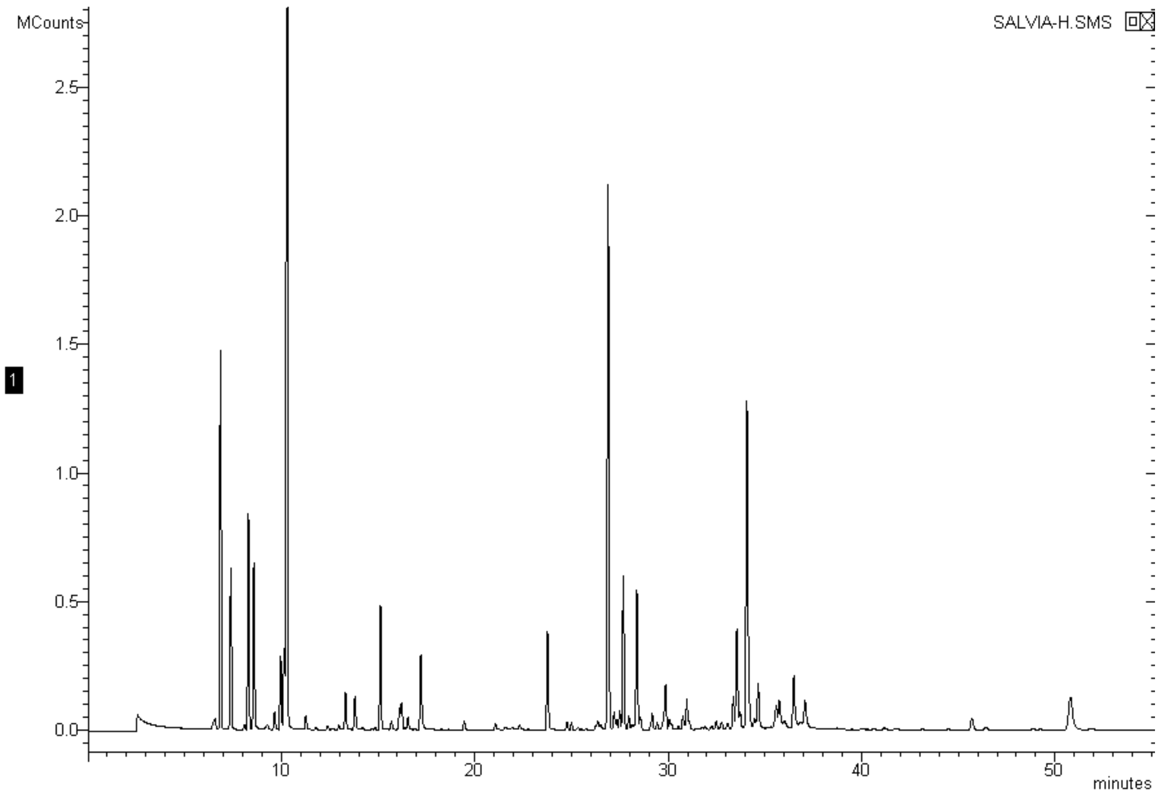
e- *Mentha aquatica*



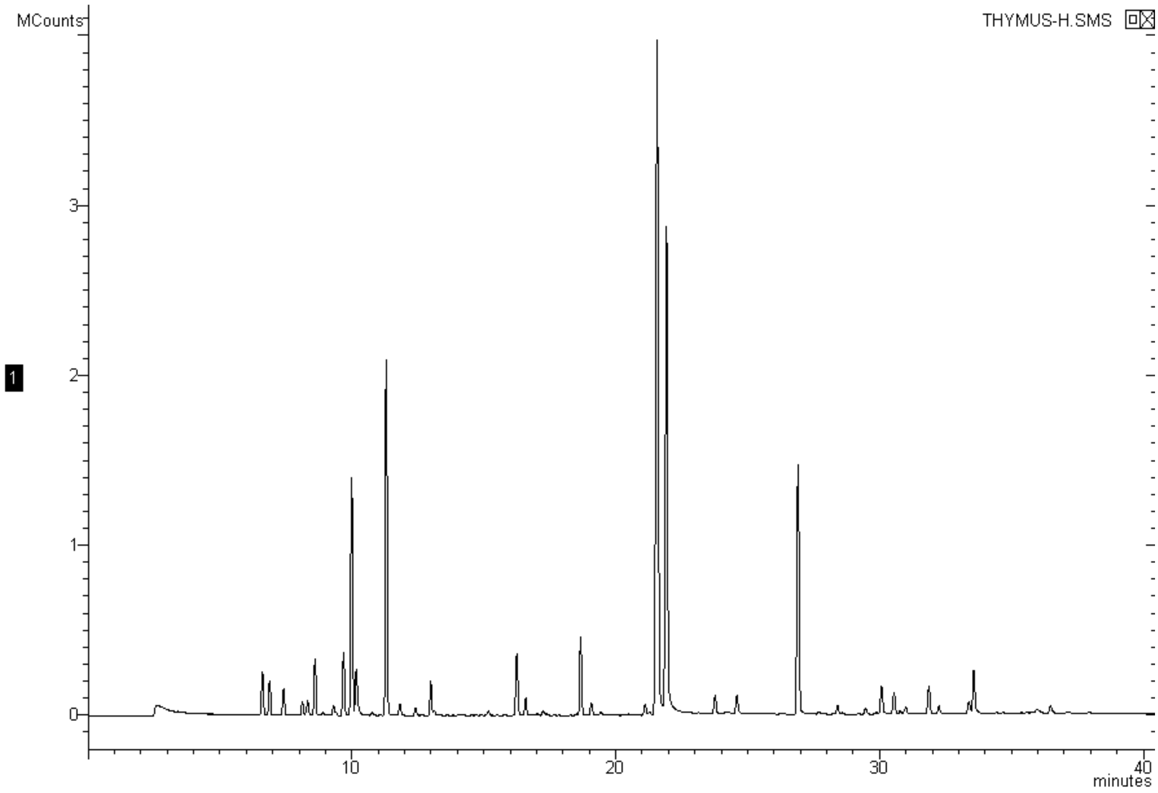
f- *Origanum syriaca*



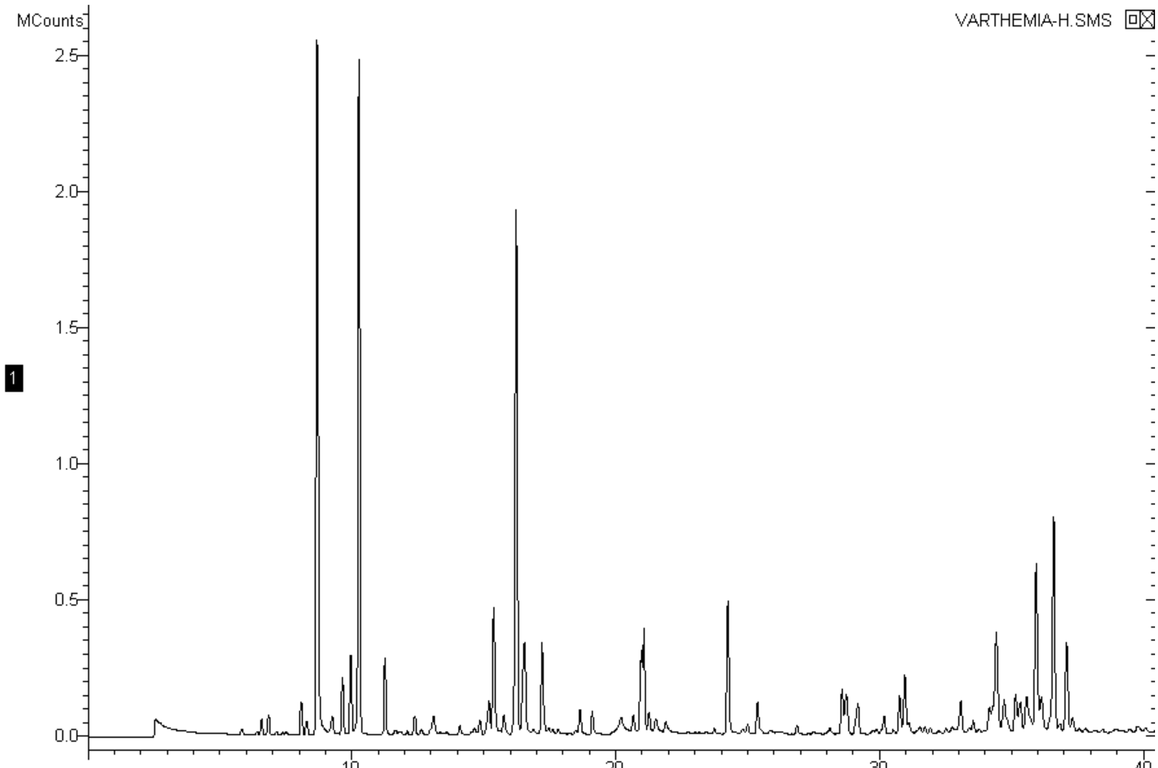
g- *Rosmarinus officinalis*



h- *Salvia palaestina*



i- *Thymus capitatus*



j- *Varthemia iphinoides*

Figure 34 (a-j): The GC-MS chromatograms of the essential oils of the tested plant species.

The chemical composition of the oils, determined by GC-MS analysis are, shown in tables (2 – 11).

Table (2) shows the chemical composition of *A. fragrantissima* oil. The GC-MS analysis revealed the identification of 15 compounds as the major (87.48% of total oil). As shown artemisia ketone, β-sesquiphellandrene and α-thujone are the main oi compounds observed with the % content: 23.98, 17.11 and 10.98 % respectively.

Table 2: Chemical composition of *A. fragrantissima* essential oil

Number	% content	CI	AI	KI	Compound
1	1.7	995	999	999	Yomogi alcohol
2	1.1	1026	1020	1024	*p*-Cymene
3	3.1	1031	1034	1040	Santolina alcohol
4	**24.0**	**1058**	**1056**	**1062**	**Artemisia ketone**
5	0.7	1080	1080	1083	Artemisia alcohol
6	11.0	1110	1101	1102	α-Thujone
7	6.4	1121	1112	1114	β-Thujone
8	1.1	1143	1137	1142	trans-Sabinol
9	7.2	1163	1169	1173	Artemisyl acetate
10	4.6	1290	1289	1290	trans-Sabinyl acetate
11	6.1	1305	1298	1299	Carvacrol
12	1.0	1486	1484	1485	Germacrene D
13	17.1	1527	1521	1522	β-Sesquiphellandrene
14	1.4	1660	1652	1653	α-Eudesmol
15	0.9	1694	1685	1685	α-Bisabolol
	87.5				

- % content : percent of the compound. – AI : Arethmatic retention index
- CI: Calculated arethmatic retention indexindex - KI: Kovats retention index

Table (3) shows the chemical composition of the *A. millefolium* essential oil. As the GC-MS result revealed that 15 (71.07%) compounds were identified. Germacrene D and 1,8-Cineole are the major compound forming 15.94 and 12.68% of the *A. millefolium* essential oil respectively.

Table (3): Chemical composition of *A. millefolium* essential oil

Number	**% content**	**CI**	**AI**	**KI**	**Compound**
1	2.2	934	932	939	α-Pinene
2	4.8	951	946	954	Camphene
3	6.13	973	969	975	Sabinene
4	1.7	980	974	979	β-Pinene
5	12.7	1034	1026	1031	1,8-Cineole
6	1.7	1057	1056	1062	Artemisia ketone
7	4.9	1151	1141	1146	Camphor
8	2.1	1259	1249	1252	Piperitone
9	**15.94**	**1484**	**1484**	**1485**	**Germacrene D**
10	4.5	1499	1500	1500	Bicyclogermacrene
11	5.2	1516	1515	1516	Sesquicineole
12	2.3	1615	1618	1619	1,10-di-epi-Cubenol
13	3.0	1634	1640	1642	epi-α(τ)-Muurolol
14	1.1	1661	1652	1653	α-Eudesmol
15	2.9	1692	1685	1685	α-Bisabolol
	71.1				

The chemical composition of *M. aquatica* essential oil is shown in table (4). As shown 5 (63.80%) compounds were identified. The results were shown that Pulegone is the major compounds observed with 50.18 % ratio.

Table 4: Chemical composition of *M. aquatica* essential oil

Number	**% content**	**CI**	**AI**	**KI**	**Compound**
1	2.4	1034	1026	1031	1,8-Cineole
2	7.2	1159	1148	1152	Menthone
3	1.6	1179	Unk	Unk	Isopulegone
4	**50.2**	**1242**	**1233**	**1237**	**Pulegone**
5	2.5	1586	1582	1583	Sabinene
	63.8				

Table (5) shows the chemical composition of *T. capitatus* essential oil. As shown 16 (88.06%) compounds were identified. The two compounds Thymol and carvacrol are the major identified compound forming 25.87 and 17.83 % of *T. capitatus* essential oil respectively.

Table 5: Chemical composition of *T. capitatus* essential oil

Number	**% content**	**CI**	**AI**	**KI**	**Compound**
1	1.3	926	924	930	α-Thujene
2	1.1	935	932	935	α-Pinene
3	1.7	990	988	990	Myrcene
4	2.0	1019	1014	1017	α-Terpinene
5	7.4	1027	1020	1024	*p*-Cymene
6	1.4	1031	1024	1029	Limonene
7	11.3	1060	1054	1059	γ-Terpinene
8	1.1	1102	1095	1096	Linalool
9	2.0	1177	1165	1169	Borneol
10	2.5	1232	1232	1235	Thymol,methyl ether
11	**25.9**	**1298**	**1289**	**1290**	**Thymol**
12	17.8	1306	1298	1299	Carvacrol
13	8.9	1423	1417	1419	β-Caryophyllene
14	1.0	1499	1500	1500	Bicyclogermacrene
15	1.0	1544	1529	1531	*E*-γ-Bisabolene
16	1.7	1586	1582	1583	Caryophyllene oxide
	88.0				

A total of 19 compounds forming 73.57 % of *V. iphinodies* essential oil were identified (table 6). 2,6-Dimethyl-3,5-heptadiene, 1,8-Cineole and Borneol were the major compounds that form 14.45, 12.66 and 10.97 % of *V. iphinodies* essential oil

Table 6: Chemical composition of *V. iphinodies* essential oil

Number	**% content**	**CI**	**AI**	**KI**	**Compound**
1	**14.4**	**992**	**NA**	**NA**	**2,6-Dimethyl-3,5-heptadiene(Oxyg-HC)**
2	1.0	1018	1014	1017	α-Terpinene
3	1.5	1026	1020	1024	*p*-Cymene
4	12.7	1034	1026	1031	1,8-Cineole
5	1.5	1059	1054	1059	γ-Terpinene
6	2.	1157	1144	1148	trans-α-Necrodol
7	11.0	1177	1165	1169	Borneol
8	1.9	1184	1174	1177	Terpinen-4-ol
9	2.0	1199	1084	1088	α-Terpineol
10	1.6	1284	1288	1290	Lavandulyl acetate
11	2.0	1287	1287	1288	Bornyl acetate
12	2.7	1360	1359	1361	Neryl acetate
13	1.2	1467	NA	NA	2-(2-butynyl)-Cyclohexanone (Oxyg
14	1.1	1521	1522	1523	δ-Cadinene
15	2.7	1608	1607	1607	β-Oplopenone
16	1.3	1638	1630	1632	γ-Eudesmol
17	4.9	1648	1638	1641	epi-α(τ)-Cadinol
18	5.2	1665	NA	NA	Unknown HC
19	2.2	1678	NA	NA	Unknown HC
	73.57				

Table (7) shows the chemical composition of the *L. dentata* essential oil as identified using GC-MS analyses. The results were shown that 18 (87.08 %) compounds were identified. As shown Linalool and 1,8-Cineole are the major two compounds in *L. dentata* essential oils, they form 24.49 and 22.22 % of the essential oil respectively.

Table 7: Chemical composition of *L. dentata* essential oil

Number	**% content**	**CI**	**AI**	**KI**	**Compound**
1	1.8	934	932	939	α-Pinene
2	0.8	973	969	975	Sabinene
3	2.4	980	974	979	β-Pinene
4	0.8	989	988	990	Myrcene
5	0.9	1011	1008	1011	δ-Carene
6	1.00	1026	1020	1024	*p*-Cymene
7	2.4	1030	1024	1029	Limonene
8	22.2	1034	1026	1031	1,8-Cineole
9	1.31	1057	1056	1062	Artemisia ketone
10	**24.5**	**1103**	**1095**	**1096**	**Linalool**
11	7.24	1151	1141	1146	Camphor
12	10.6	1177	1165	1169	Borneol
13	2.5	1184	1174	1177	Terpinen-4-ol
14	2.2	1192	1191	1192	Hexyl butanoate
15	1.7	1199	1084	1088	α-Terpineol
16	1.6	1250	1254	1257	Linalool acetate
17	1.6	1454	1454	1456	*E*-β-Farnesene
18	1.4	1691	1685	1685	α-Bisabolol
	87.08				

The chemical composition of *O. syriaca* essential oil as identified by GC-MS analyses is shown in table (8). The analyses revealed that 13 (82.55%) compounds were identified. As shown Carvacrol and Thymol are the major component forming 40.88 and 27.54% of *M. syriaca* essential oilrespectively.

Table 8: Chemical composition of *O. syriaca* essential oil

Number	**% content**	**CI**	**AI**	**KI**	**Compound**
1	0.9	925	924	930	α-Thujene
2	0.69	934	932	939	α-Pinene
3	2.2	989	974	979	β-Pinene
4	1.0	1018	1014	1017	α-Terpinene
5	1.2	1026	1020	1024	*p*-Cymene
6	0.7	1034	1026	1031	1,8-Cineole
7	3.2	1059	1054	1059	γ-Terpinene
8	1.0	1072	1065	1070	cis-Saninene hydrate
9	1.2	1184	1174	1177	Terpinen-4-ol
10	27.5	1298	1289	1290	Thymol
11	**40.9**	**1307**	**1298**	**1299**	**Carvacrol**
12	0.8	1422	1417	1419	β- Caryophyllene
13	1.2	1586	1582	1583	Caryophyllene oxide
	82.6				

Table (9) shows the chemical composition of the *R. officinalis* essential oil as revealed by GC-MS analyses. The results were shown that 1,8-Cineole and α-Pinene were the major component of *R. officinalis* essential oil forming 29.27 and 21.40 % of the oil respectively.

Table 9: Chemical composition of the *R. officinalis* essential oil

Number	**% content**	**CI**	**AI**	**KI**	**Compound**
1	21.4	934	932	939	α-Pinene
2	6.6	951	946	954	Camphene
3	3.5	980	974	979	β-Pinene
4	1.2	989	988	990	Myrcene
5	4.3	1026	1020	1024	*p*-Cymene
6	4.9	1031	1024	1029	Limonene
7	**29.3**	**1035**	**1026**	**1031**	**1,8-Cineole**
8	2.5	1102	1095	1096	Linalool
9	6.8	1152	1141	1146	Camphor
10	1.6	1177	1165	1169	Borneol
11	1.2	1213	1204	1205	Verbenone
12	4.2	1287	1287	1288	Bornyl acetate
13	1.8	1586	1582	1583	Caryophyllene oxide
	89.2				

A total of 21 compounds forming 84.63 % of *S. palaesteina* essential oil were identified (table 10). As shown 1,8-Cineole and β-Caryophyllene were the major compounds that form 24.29and 11.10 % of *S. palaeseina* essential oil.

Table 10: Chemical composition of the *S. palaesteina* essential oil.

Number	**content%**	**CI**	**AI**	**KI**	**Compound**
1	6.6	934	932	939	α-Pinene
2	2.9	951	946	954	Camphene
3	4.1	979	974	979	β-Pinene
4	3.0	989	988	990	Myrcene
5	1.4	1026	1020	1024	*p*-Cymene
6	1.5	1030	1024	1029	Limonene
7	**24.3**	**1034**	**1026**	**1031**	**1,8-Cineole**
8	2.4	1151	1141	1146	Camphor
9	1.6	1199	1084	1088	α-Terpineol
10	2.0	1349	1346	1349	α-Terpinyl acetate
11	11.1	1422	1417	1419	β-Caryophyllene
12	3.3	1441	1439	1441	Aromadendrene
13	2.99	1458	1452	1454	α-Humulene
14	1.0	1494	1496	1496	Viridiflorene
15	0.9	1521	1522	1523	δ-Cadinene
16	0.7	1581	1577	1578	Spathulenol
17	2.4	1586	1582	1583	Caryophyllene oxide
18	8.1	1599	1592	1592	Viridiflorol
19	1.1	1614	1608	1608	Humulene epoxide II
20	1.4	1662	1668	1669	14-hydroxy-9-epi-(*E*)-Caryophyllene
21	1.8	2054	2050	2057	Manool
	84.6				

A total of 21 compounds forming 90.11 % of *J. phoenica* essential oil were identified (table 11). As shown Limonene was the major compounds that form 17% of *Juniperus phoenica* essential oil.

Table 11: Chemical composition of the *Juniperus phoenica* essential oil

Number	**% content**	**CI**	**AI**	**KI**	**Compound**
1	1.5	925	921	926	Tricyclene
2	1.7	934	932	939	α-Pinene
3	0.5	951	946	954	Camphene
4	15.5	973	969	975	Sabinene
5	9.4	989	988	990	Myrcene
6	1.9	1018	1014	1017	α-Terpinene
7	**17.0**	**1031**	**1024**	**1029**	**Limonene**
8	0.63	1046	1044	1050	(E)-β-Ocimene
9	2.5	1059	1054	1059	γ-Terpinene
10	1.4	1087	1086	1088	Terpinolene
11	0.7	1121	1112	1114	β-Thujone
12	1.7	1184	1174	1177	Terpinen-4-ol
13	13.7	1287	1287	1288	Bornyl acetate
14	8.5	1291	1289	1290	trans-Sabinyl acetate
15	2.4	1521	1522	1523	δ-Cadinene
16	4.5	1553	1548	1549	Elemol
17	2.0	1581	1592	1592	Viridiflorol
18	0.6	1608	1607	1607	β-Oplopenone
19	0.6	1648	1638	1641	epi-α(τ)-Cadinol
20	1.0	1650	1640	1642	epi-α(τ)-Muurolol
21	2.4	1661	1652	1654	α-Cadinol
	90.1				

The Chemotype of the tested plant species

The chemotype of the tested plant are shown in table 12. The chemotype of the plant species were determind depending on the compund that have the highest percentage in the oils (Principal).

Table 12: The chemotype of the tested plant species.

Plant Species	Family	Common name	Chemotype
Achillea fragrantissmia Forssk	*Asteraceae*	Yarrow	**Artemisia ketone**
Achillea millefolium L.	*Asteraceae*	Yarrow	**Germacrene D**
Juniperus phoenica L.	*Cupressaceae*	Juniper	**Limonene**
Lavandulla dentata	*Lamiaceae*	Lavander	**Linalool**
Mentha aquatica L.	*Lamiaceae*	Menth	**Pulegone**
*Origanum syriac*a (L.) Rafin.	*Lamiaceae*	Origano	**Carvacrol**
Rosmarinus officinalis L.	*Lamiaceae*	Rosemary	**1,8-Cineole**
Slavia palaestina Benth.	*Lamiaceae*	Sage	**1,8-Cineole**
Thymus capitatus (L.) Hoffmanns. & Link	*Lamiaceae*	Thyme	**Thymol**
Varthemia iphinoides Boiss. & Blanche	*Asteraceae*	Common Varthemia	**2,6-Dimethyl-3,5-heptadien**

CHAPTER FOUR

DISCUSSION

Essential Oil and Extract Preparation

The study aimed to quantify and compare the antimicrobial and antioxidant activity of 10 selected aromatic Jordanian medicinal plants essential oils. These plant species are commonly used in traditional medicine in Jordan. Among the various extraction techniques the solvent extraction technique was preferred for crude extraction and steam distillation was used for essential oil extraction in this study. The active plant constituents are affected by the used extraction method (Shaalan, *et al*. 2005), the plant part used for extraction, type of extraction solvent, plant material to solvent ratio and extraction time are some other important factors affecting the activity of an extract (Taylor, *et al*., 1995 and Padmaja, *et al*., 1995). The traditional techniques of solvent extraction of plant materials are mostly based on the correct choice of extraction solvents and the use of heat or/and agitation to increase the extraction of the desired constituents and improve extraction procedure. Usually the extraction technique requires longer extraction time and heating running a severe risk of degradation for most of the phytochemicals (Mandal, *et al*. 2007).

A preliminary study was carried out in order to display the influence of some parameters used in extraction including solvent concentration, plant material to solvent ratio and extraction time. It was found that the extraction of plant for 5 minutes is significantly comparable to overnight extraction , that finding give an indication about the nature of the active constituents of the tested plant. The differences in the chemical nature and concentration of phyochemicals made it difficult to optimize extraction parameters for all plants used bioactivity screening studies.

To avoid the problem it is important to use standard procedure for extraction of plant used in biological activity screening studies, this is achieved by using different solvents to extract different plant constituents. Solvent types can significantly affect the potency of extracted plant compounds (Ouf *et al*. 1994 and Eloff, 1998). Many biologically active natural materials have been extracted with organic solvents. However, some of the organic solvents are believed to be toxic, and the extraction conditions are often harsh. A simple method using ethanol instead of methanol for the extraction of phenolic compounds is the preferance frequently in the literature (Li, *et al*. 2006).

The extraction of essential oils using steam distillation technique made it easier to compare the biological activity of these oils. The essential oils have less phytochemicals comparing to crude extract. The use of steam distillation provides good way to avoid the effect of polarity and concentration of solvents. However, it is easier to identify the phytochmicals using essential oils than crude extract. There is an increase attitude toward the use of essential oils in screening of medicinal plants biological activity (Tepe, *et al.,* 2007, Sokovic *et al.,* 2006 and Lopez *et al.* 2005).

Antimicrobial activity of plant extracts and essential oils

The antibacterial activity of plant extracts and essential oils was performed against four bacterial species. *E. coli* and *P. auregenosa* are gram negative bacteria, the other two species *B. subtilis* and *S. aureus* are gram positive bacteria. All the tested essential oils and different organic extract (figures 1 – 4) possessed antibacterial activity and inhibit the growth of all tested bacterial species. The essential oils of all medicinal plant possess significantly higher activity comparing to other organic extract which may refer to the type and concentration of phyochemicals found presence in these oils.

The essential oils and organic extract showed variation in antibacterial activity against different bacteria species. The essential oils and *V. iphinoides* showed higher activity against *S. aureus* comparing to standard antibiotic (RIZD = 281.48 ± 12.47%), while *M. aquatia* essential oil showed significantly higher RIZD (188.64 ± 8.7 %)compating to other essential oils against *E. coli* comparing. The variations in bacterial response to essential oils may refer to the structural difference of bacteria and the mode of action of the essential oils against these bacterial species.

The antifungal activity of plant extracts and essential oils was performed against five fungal species. These species were chosen according to many criteria, *C. albicans* a pathogenic yeast and *A. niger* and *M. mehei* that cause food contamination, *T. mentagrophytes* a human dermatophytes and *F. oxysporium* a plant pathogen. All the tested essential oils and different organic extract (figures 5–6) possessed antifungal activity and inhibit the growth of all tested fungal species. The essential oils of all medicinal plant possess significantly higher activity comparing to other organic extract which may refer to the type and concentration of phyochemicals found presence in these oils.

It was observed that the effect of plant extracts and essential oils on *F. oxysporium* was significantly lower than the effect on other tested fungi, this may due that fact that this fungus is a plant pathogen and may have certain mechanisms to resist the effect of plant originated compounds.

The methanolic extracts the tested plants possessed higher activity comparing to ethanolic and ether extract, this result is coup with Ouf *et al*. (1994) results. This is referring to the nature and polarity of the antimicrobial active compounds which is highly dissolved in methanol the highest polar compound between the used organic solvent.

Comparison between results reported about antibacterial properties of different essential oils and plant extracts is very difficult due to the variation in methods of antimicrobial effects evaluation, source of essential oil and different species of used microbes (Arab and Ettehad, 2008).

Several studies have been carried out on antimicrobial effects of plants and they have demonstrated that thymol and carvacrol are the most important components that are responsible for antimicrobial properties. Kim *et al*. (1995) studied the antibacterial effects of carvacrol on *Salmonella typhimurium* and its rifampin resistant genus. They found that carvacrol showed powerful bactericidal effect against rifampin-resistant genus. In another study, Karman *et al*. (2001) showed powerful bacteriostatic effect of Thymus revolatus essential oil on *staphylococcus aureus*. They illustrated high amount of carvacrol in essential oil is possible reason of this effects. Like previous study, high amount of carvacrol in the essential oil was mentioned as reason of powerful bactericidal effect of volatile oil. Similar results were obtained in studies about effects of Thyme and compounds of carvacrol and thymol on *Shigella sonnei*, and *Shigella flexneri* (Bagamboula *et al*, 2004). Delgado *et al*. (2004) showed that simultaneous application of thymol and ρ-cymen resulted in a greater antibacterial effect than when the compounds were used separately. Therefore, the presence of high amount of thymol, carvacrol and ρ-cymene in plant essential oils, could be considered as a reason of antimicrobial effects of the plant.

Minimal Inhibitory Concentration (MIC)

The minimal inhibitory concentration (MIC) is an assay used to study the effect of certain compound against microorganisms. It was performed by culturing of each tested microorganism in liquid culture containing different concentration of plant essential oil, after incubation at optimum temperature the concentration that prevent growth recorded as MIC. This assay provide clear picture about the activity of tested essential oils. As shown in table (1) the tested essential oils have wide range of MIC, *T. capitatus* showed very low MIC (2.5- 20µg/ml)comparing to standards antibiotic and antifungal(200 – 750 µg/ml) that gave him stronger activity, this result agreed with the results of Rota *et al*.(2008).

All tested essential oils except *J. phoenica* and *M. aquatic* showed stronger activity (lower MIC) comparing to standard antibiotic and antifungal. The variations in MIC for the different plant and against different bacterial species are due to the reasons mention above, about the structural differences and the qualitative and quantitative variation between oils.

It is interesting to point out that the bacteria demonstrating the biggest inhibition zones by diffusion method are not always the ones that present the lowest MIC and MBC values. In fact, the diameter of the growth inhibition zone is affected by the oil solubility and volatility (Hernandez *et al*., 2005). The result of our study showed that there is no direct relationship between the RIZD and MIC.

The observed result show low MICs for bacterial species comparing to that for fungi, this finding due the differences in structure and resistance mechanisms between prokaryotes and eukaryotes. The MICs for gram negative and gram positive species varied between essential oils.

Effect of essential oils on spore germination

The essential oils reduce the percentage of germination spore at concentration dependent manner (figures 10 – 19). All essential oils completely inhibit spore germination at different concentration. The inhibition of spore germination was observed to be concentration dependent. The germination percentage at the MIC of the essential oils showed more than 90% spore germination inhibition. These results coup with Udomsilp *et al*. (2009) result about the efficacy tests of two essential oils that undertaken by mycelium growth and spore germination inhibition under in vitro conditions against rice pathogenic fungi. The experimental results showed that cassia oil at concentration 2.0%(v/v) completely inhibited mycelium growth and spore germination on *Alternaria brassicicola, Aspergillus flavus, Bipolaris oryzae, Pyricularia oryzae, Bipolaris oryzae,Fusarium moniliforme, Fusarium proliferatum* and *Rhizoctonia solani.* Farid *et al*. (2002), reported that the efficacy of medicinal plants against various species of pathogenic fungi was dependent on the origin of the medicinal plant and the sensitivity of the target fungi.

The inhibition of spore germination by low concentration of plant essential oils give the essential oils a good chance to be used for inhibition of fungal growth. These essential oils may replace antifungal drugs due to the fact of the side effect of these drugs.

Evaluation the mode of action of plant essential oils on microorganisms

Few investigations were concerned with the mode of action of essential oils on the microorganisms. The effect of plant compound on microorganisms include inhibition of amino acid synthesis (Mori, *et al*., 1987), inhibition of respiration (Harguchi, *et al*., 1996), breakdown of cell membrane (Kubo and Taniguchi, 1988)

and inhibition of cell wall synthesis (Sabanero, *et al*., 1995) in addition to the inhibition of spore germination.

The addition of some cellular constituents to the culture media may substitute the effected cellular components during the antimicrobial assay. It was observed that addition of fatty acids inhibit the effect of essential oils on the fungi (Figure 20). There was no inhibition for the growth with the presence of fatty acid in the media. This may refer to the effect of essential oils on the lipid component of the cells. Fatty acid may substitute the biosynthesis of lipid, or the essentail oils act on the added fatty acid and not affected the fungi.

Antioxidant activity

Due to the chemical complexity of plant extracts, different methods are required to assess their antioxidant activity. In this study, many tests were selected to evaluate the primary and the secondary step of oxidation; the ability to scavenge free radicals and the capacity to inhibit lipid oxidation. The reductive potential was also measured.

The antioxidant activity of essential oils is important biological property of great interest because they may preserve foods from the toxic effects of oxidants (Maestri *et al.*, 2006*)* Moreover, essential oils being also able of scavenging free radicals may play an important role in some disease prevention such as brain dysfunction, cancer, heart disease and immune system decline. Increasing evidence has suggested that these diseases may result from cellular damage caused by free radicals (Kamatou and Viljoen, 2010). If essential oils are able to scavenge some free radicals, they can also act as anti-inflammatory agents, because one of the inflammatory responses is the oxidative burst that occurs in diverse cells (monocytes, neutrophils, eosinophils, and macrophages). Phagocytosis of bacteria, which occurs

during inflammation, is accompanied by a dramatic increase in oxygen consumption resulting in the formation of superoxide anion radical ($O2^{\cdot -}$) which is quickly converted to hydrogen peroxide (H_2O_2),spontaneously, or by the enzyme superoxide dismutase. Hydrogen peroxide can then also be reduced by transition metal ions generating the hydroxyl radical ($HO^{\cdot}$), one of the strongest oxidizing agents that can rapidly react with polyunsaturated fatty acids, resulting the production of peroxyl radicals ($ROO^{\cdot}$). Peroxide hydrogen can also oxidise halide ions (Cl^-) to hypochlorous acid (HOCl), which is a strong oxidant that can react with amines producing chloramines, some of them being very toxic (Chen and Schopfer, 1999)

DPPH Scavenging Activty

The free radical scavenging activities of the plant essential oils were investigated using DPPH assay (figures 20–22). Reduction of the DPPH radical, which accepts an electron of hydrogen radical to become a stable diamagnetic molecule, was determined by the decrease in its absorbance by antioxidants. The results were shown that the reaction followed a concentration dependent pattern. The free radical scavenging activity of essential oils of *T. capitatus, J. Phoenica*, *R. officinalis* and *L. dentata* was superior to vitamin C. Activity might be related to its phenolic content like phenolic acids polyphenols as reported in a previous study by Mimica-Dukic *et al*. (1999). The type of essential oils phytochmical and their concentration affect the antioxidant properties of the oil.

It is so difficult to compare between the DPPH scavenging activity of essential oils for the same plant collected from different location and at different seasonal conditions. The type and amount of active constituents varies among species from different location thus the plant know are identified according to their chemo-type

The DPPH assay is based on the measurement of the relative inhibition of the essential oils tested at various concentrations. Essential oil that are able to convert the color of purple DPPH solution to yellow can be considered as antioxidant and there for radical scavenging (Hinneburg et al., 2006).

Nitrogen Oxide (NO) Scavenging Activty:

Nitric oxide (NO) is a potent inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities (Hagerman *et al.*, 1998). Suppression of released NO may be partially attributed to direct NO scavenging, as the essential oils decreased the amount of nitrite generated from the decomposition of sodium nitroprusside in vitro. The scavenging of NO by the essential oils was increased in dose dependent manner. Figures 23-25 illustrate a significant decrease in the NO radical due to the scavenging ability of essential oils and Rutin. The essential oil of *L. dentata* showed maximum activity of 30 µg /ml, where as rutin at the same concentration exhibited significantly lower inhibition.

Reducing Power

The reducing powers of the plants essential oils of and standards were performed. The reducing power of the oils increased with increasing essential oils concentration. There is variation in the reducing power of essential oils of *T. capitatus, J. phoenica, R. officinalis* and *L. dentata*, at all concentrations (5 - 30µg/ml), exhibited higher activities than the control. While the essential oils of *M. syriaca, A. millefoulim* and *A. fragrantissima* possessed significantly lower activity comparing BHT. Some reports have noted that the antioxidant effect is concomitant

with the development of reducing power (Duh et al., 2001). Therefore, the antioxidant activity of *T. capitatus, J.phoenica, R. officinalis* and *L. dentata* may be related to their reducing power.

Reducing power assay measures the electron-donating capacity of an antioxidant. The reducing properties are associated with the presence of reductones, which have been shown to exhibit antioxidant action by breaking the chain reactions by donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation (Matsushige *et al*., 1996). Being good electron donors, phenolic compounds show the reducing power and have ability to convert the ferric ion Fe^{3+} to ferrous ion Fe^{2+} by donating an electron (Shon *et al*., 2004). Increasing absorbance at 700 nm indicates an increasein reductive ability. The ability to reduce Fe(III) may be attributed to hydrogen donation from phenolic compounds (Shimada *et al*., 1992), which is also related to the presence of reductant agent.

Lipid Preoxidation

The lipid peroxidation assay was performed for tested plant essential oils (figure 32), the essential oils of *R. officinals, J. phoenica* and *L dentata* possessed stronger antioxidant activity comparing to vitamin E. The essential oil of *V. iphinoides* showed similar lipid antioxidant activity comparing with vitamin E, while *A. millefolim* and *A. fragrantissma* showed significantly lower activity. The variations in the activity refer to the phyochemical composition of each essential oils and the ratio of these phytochemicals. Polyphenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity and phenolic compounds are also known as powerful chain breaking antioxidants (Shahidi &

Wanasundara, 1992). The antioxidant activities of diterpenoids were studied and they were found to be highly active (Kabouche, *et al.*, 2007).

It had been defined as the oxidative deterioration of polyunsaturated lipids. Initiation of a peroxidation sequence in a membrane or polyunsaturated fatty acid is due to abstraction of a hydrogen atom from the double bond in the fatty acid. The free radical tends to stabilized by a molecular rearrangement to produce a conjugated diene. This reacts with oxygen molecule to give a peroxy radical. Peroxy radical can abstract a hydrogen atom from another molecule to give lipid hydro peroxide (Sathisha *et al*,2011).

Superoxide anion scavenging activity

The superoxide anion scavenging activity was performed the results were shown that all the essential oils possess scavenging activity in concentration dependent manner (figure 26-28). The essential oils possessed variation in the scavenging activity, for example, *T. capitatus, J.phoenica, R. officinalis* and *L. dentata* possess scavenging activity at significantly lower concentration comparing to standard. The chemical composition of the essential oils showed that they contain variety of compound with different ratio. The differences between oils may be due to these differences in addition to the role of agonistic compound that may play important role on the activity.

The superoxide anion scavenging activity of *Thymus* oils were evaluated by the enzymatic hypoxan thine/xanthine oxidase system . In *Thymus zygis* ssp. *sylvestris* prevailed thymol or carvacrol, whereas in *Thymus capitellatus* oils, borneol and 1,8-cineole dominated. Such results seem to indicate that the phenolic compounds are not the only ones able to scavenge superoxide anion (Dandlen *et al*. 2010)

Phytochemical analysis of essential oils

The chemical composition of plant essential oils was performed using MS-GC analysis (table 2-11). The ratio of identified compound range between 63.80 % for *M. aquatica* and 90.11% for *J. phoenica.* Each essential oil showed a different chemical composition. The ratio of some molecule found in different plant varied according to the species. The time of collection of plant effect the nature and rtio of the constituents.

The percentage of the components present in essential oils varies greatly. Major constituents can form up to 85% of the essential oils, while the remaining components can be present in only trace amounts (Miguel, 2010)]. The aroma of each oil results from the combination of the aromas of all components, and even minor oil constituents may have major organoleptic roles to play.

The chemotype of the tested plant are shown in table 12. The chemotype may be varied depending on many factors such as geographical variations, seasonal variation, and genetic variations.

COCLUSION AND RECOMMENDATION

Medicinal plants essential oils have important attention of researcher recently due their biological activity. Antimicrobial and antioxidant activity of 10 plant essential oils were investigated in this study. The essential oils were extracted using steam distillation method which is good method for isolation essential oils. The essential oils possessed significantly high antimicrobial and antioxidant activity comparing to standard.

All essential oils showed the antimicrobial activity in concentration dependent manner. The MIC of *T. capitatus* and *M. syriaca* showed stronger antimicrobial activity comparing to antibiotic and antifungal. These essential oils may used in the treatment of bacterial and fungal infections.

The antioxidant activity was performed using different methods. All plants essential oils possessed antioxidant activity with all tests. The stronger antioxidant activity of plants put them in priority to be used as natural antioxidant in food industries. These plants not have the side effect and carcinogenic effect of synthesized antioxidant used in food industry.

The essential oil constituent varies among plant essential oils.There is a clear relationship between the chemical composition and the antimicrobial and antioxidant activity. Some oil constituents may play antagonistic role in the biological activity.

It is recommended to:

1- Isolate and separate the constituents of essential oils and study the biological activity of each compound.

2- Screen the effect of seasonal and geographical variations on essential oil activity.

3- Study the synergistic effect of mixed oils.

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اختبار الفعالية المضادة للميكروبات والفعالية المضادة للأكسدة للزيوت المتطايرة المعزولة من بعض النباتات الطبية العطرية الأردنية

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الملخص

هدفت هذه الدراسة الى غربلة الفعالية المضادة للميكروبات والفعالية المضادة للاكسدة اضافة الى التركيب الكيميائي للزيوت الطيارة لعشرة نباتات عطرية طبية اردنية. حيث استخلصت الزيوت المتطايرة لهذه النباتات والتي جمعت من مناطق مختلفة بطريقة التقطير البخاري.

استخدمت طريقتي حفر الاجار واقل تركيز مثبط لدراسة الفعالية المضادة للميكروبات. حيث اظهرت جميع الزيوت فعالية مضادة للميكروبات وبدرجات متفاوتة ضد جميع الميكروبات تحت الدراسة. وجد ان الزيت الطيار لنبات *T. capitatus* أظهر فعالية اعلى مقارنة مع المضاد الحيوي المعياري وباقي الزيوت الطيارة.كما تم دراسة اثر الزيوت الطيارة على انبات السبورات الفطرية حيث تبين ان الزيوت الطيارة ثبطت انبات السبورات الفطرية وكان هذا التثبيط معتمدا على تركيز الزيوت. حيث كان هناك اكثر من ٩٠% تثبيط لانبات السبورات عند اقل تركيز مثبط لنمو الفطريات. كما لم يلاحظ اي انبات للسبورات عند تراكيز اعلى من اقل تركيز مثبط.

تم اختبار الفعالية المضادة للاكسدة للزيوت الطيارة باستخدام طرق تقدير مختلفة مثل DPPH scavenging و NO scavenging و Lipid peroxidation. وقد اظهرت النتائج ان جميع الزيوت الطيارة عملت كمضادات اكسدة باستخدام واحدة او أكثر من هذه الطرق. كما ووجد ان هذه الفعالية المضادة للاكسدة معتمدة على التركيز. وقد حددت التراكيب الكيمائية للزيوت الطيارة باستخدام تقنية كروماتوغرافيا الغاز – مطياف الكتلة (GC-MS).

تم تعريف عدد من المركبات والتي لهما علاقة مباشرة مع فعالية الزيوت الطيارة المضادة للميكروبات والاكسدة. حيث اظهرت النتائج احتواء الزيوت الطيارة على مركبات فينولية ذات خصائص مضادة للميكروبات والاكسدة. وعلى سبيل المثال وجد ان الزيوت الطيارة لنباتي *T. capitatus* و *M. syriaca* تحتوي نسب عالية من مركبات فينولبة مثل الثايمول والكارفاكرول والتي لها فعالية مضادة للميكروبات.